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(57) Abstract

The present invention relates to novel members of the Tumor Necrosis Factor family of receptors. The invention provides isolated nucleic acid molecules encoding human TR8 receptors. TR8 polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. The inventon further relates to screening methods for identifying agonists and antagonists of TR8 receptor activity. Also provided are diagnostic methods for detecting disease states related to the aberrant expression of TR8 receptors. Further provided are therapeutic methods for treating disease states related to aberrant proliferation and differentiation of cells which express the TR8 receptors.

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Human Tumor Necrosis Factor Receptor-Like Protein 8

Field of the Invention

The present invention relates to novel members of the Tumor Necrosis Factor (TNF) receptor family. More specifically, isolated nucleic acid molecules are provided encoding a human TNF receptor-related protein, referred to herein as the TR8 receptor of FIGS. 1A-C, having considerable homology to human type 2 TNF receptor (TNF-RII). TR8 polypeptides are also provided. Further provided are vectors, host cells and recombinant methods for producing the same. The invention also relates to both the inhibition and enhancement of the activity of TR8 receptor polypeptides and diagnostic methods for detecting TR8 receptor gene expression.

Background of the Invention

Human tumor necrosis factors α (TNF-α) and β (TNF-β or lymphotoxin) are related members of a broad class of polypeptide mediators, which includes the interferons, interleukins and growth factors, collectively called cytokines (Beutler, B. and Cerami, A., *Annu. Rev. Immunol.*, 7:625-655 (1989)).

Tumor necrosis factor (TNF-α and TNF-β) was originally discovered as a result of its anti-tumor activity, however, now it is recognized as a pleiotropic cytokine playing important roles in a host of biological processes and pathologies. To date, there are ten known members of the TNF-related cytokine family, TNF-α, TNF-β (lymphotoxin-α), LT-β, TRAIL and ligands for the Fas receptor, CD30, CD27, CD40 (also known as CDw40), OX40 and 4-1BB receptors. These proteins have conserved C-terminal sequences and variable N-terminal sequences which are often used as membrane anchors, with the exception of TNF-β. Both TNF-α and TNF-β function as homotrimers when they bind to TNF receptors.

TNF is produced by a number of cell types, including monocytes, fibroblasts, T-cells, natural killer (NK) cells and predominately by activated macrophages. TNF-

α has been reported to have a role in the rapid necrosis of tumors, immunostimulation, autoimmune disease, graft rejection, producing an anti-viral response, septic shock, cerebral malaria, cytotoxicity, protection against deleterious effects of ionizing radiation produced during a course of chemotherapy, such as denaturation of enzymes, lipid peroxidation and DNA damage (Nata *et al., J. Immunol. 136(7)*:2483 (1987)), growth regulation, vascular endothelium effects and metabolic effects. TNF-α also triggers endothelial cells to secrete various factors, including PAI-1, IL-1, GM-CSF and IL-6 to promote cell proliferation. In addition, TNF-α up-regulates various cell adhesion molecules such as E-Selectin, ICAM-1 and VCAM-1. TNF-α and the Fas ligand have also been shown to induce programmed cell death.

TNF-β has many activities, including induction of an antiviral state and tumor necrosis, activation of polymorphonuclear leukocytes, induction of class I major histocompatibility complex antigens on endothelial cells, induction of adhesion molecules on endothelium and growth hormone stimulation (Ruddle, N. and Homer, R., *Prog. Allergy 40:*162-182 (1988)).

Both TNF-α and TNF-β are involved in growth regulation and interact with hemopoietic cells at several stages of differentiation, inhibiting proliferation of various types of precursor cells, and inducing proliferation of immature myelomonocytic cells. Porter, A., *Tibtech 9*:158-162 (1991).

Recent studies with "knockout" mice have shown that mice deficient in TNF-β production show abnormal development of the peripheral lymphoid organs and morphological changes in spleen architecture (reviewed in Aggarwal *et al., Eur Cytokine Netw, 7(2):*93-124 (1996)). With respect to the lymphoid organs, the popliteal, inguinal, para-aortic, mesenteric, axillary and cervical lymph nodes failed to develop in TNF-β -/- mice. In addition, peripheral blood from TNF-β -/- mice contained a three fold reduction in white blood cells as compared to normal mice. Peripheral blood from TNF-β -/- mice, however, contained four fold more B cells as

compared to their normal counterparts. Further, TNF-β, in contrast to TNF-α, has been shown to induce proliferation of EBV-infected B cells. These results indicate that TNF—β is involved in lymphocyte development.

The first step in the induction of the various cellular responses mediated by TNF-α or TNF-β is their binding to specific cell surface or soluble receptors. Two distinct TNF receptors of approximately 55-KDa (TNF-RI) and 75-KDa (TNF-RII) have been identified (Hohman *et al., J. Biol. Chem., 264:*14927-14934 (1989)), and human and mouse cDNAs corresponding to both receptor types have been isolated and characterized (Loetscher *et al., Cell, 61*:351 (1990)). Both TNF-Rs share the typical structure of cell surface receptors including extracellular, transmembrane and intracellular regions.

These molecules exist not only in cell bound forms, but also in soluble forms, consisting of the cleaved extra-cellular domains of the intact receptors (Nophar *et al., EMBO Journal, 9 (10):*3269-76 (1990)) and otherwise intact receptors wherein the transmembrane domain is lacking. The extracellular domains of TNF-RI and TNF-RII share 28% identity and are characterized by four repeated cysteine-rich motifs with significant intersubunit sequence homology. The majority of cell types and tissues appear to express both TNF receptors and both receptors are active in signal transduction, however, they are able to mediate distinct cellular responses. Further, TNF-RII was shown to exclusively mediate human T-cell proliferation by TNF as shown in PCT WO 94/09137.

TNF-RI dependent responses include accumulation of C-FOS, IL-6, and manganese superoxide dismutase mRNA, prostaglandin E2 synthesis, IL-2 receptor and MHC class I and II cell surface antigen expression, growth inhibition, and cytotoxicity. TNF-RI also triggers second messenger systems such as phospholipase A, protein kinase C, phosphatidylcholine-specific phospholipase C and sphingomyelinase (Pfefferk *et al., Cell, 73:*457-467 (1993)).

Several interferons and other agents have been shown to regulate the expression of TNF receptors. Retinoic acid, for example, has been shown to induce the production of TNF receptors in some cells type while down regulating production in other cells. In addition, TNF-α has been shown to affect the localization of both types of receptor. TNF-α induces internalization of TNF-RI and secretion of TNF-RII (reviewed in Aggarwal *et al., supra*). Thus, the production and localization of both TNF-Rs are regulated by a variety of agents.

Both the yeast two hybrid system and co-precipitation and purification have been used to identify ligands which associate with both types of the TNF-Rs (reviewed in Aggarwal *et al., supra* and Vandenabeele *et al., Trends in Cell Biol.* *5*:392-399 (1995)). Several proteins have been identified which interact with the cytoplasmic domain of a murine TNF-R. Two of these proteins appear to be related to the baculovirus inhibitor of apoptosis, suggesting a direct role for TNF-R in the regulation of programmed cell death.

Summary of the Invention

The present invention provides isolated nucleic acid molecules comprising polynucleotides encoding a TR8 receptor having the amino acid sequence shown in FIGS. 1A-C (SEQ ID NO:2) or the amino acid sequence encoded by the cDNA clone encoding the TR8 receptor deposited in a bacterial vector as ATCC Deposit Number 97956 on March 13, 1997. The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of TR8 polypeptides or peptides by recombinant techniques.

The invention further provides isolated TR8 polypeptides having amino acid sequences encoded by the polynucleotides described herein.

The present invention also provides a screening method for identifying compounds capable of enhancing or inhibiting a cellular response induced by TR8 receptors, which involves contacting cells which express TR8 receptors with the candidate compound, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made in absence of the candidate compound; whereby, an increased cellular response over the standard indicates that the compound is an agonist and a decreased cellular response over the standard indicates that the compound is an antagonist.

In another aspect, a screening assay for agonists and antagonists is provided which involves determining the effect a candidate compound has on the binding of cellular ligands to TR8 receptors. In particular, the method involves contacting TR8 receptors with a ligand polypeptide and a candidate compound and determining whether ligand binding to the TR8 receptors is increased or decreased due to the presence of the candidate compound.

The invention further provides a diagnostic method useful during diagnosis or prognosis of a disease states resulting from aberrant cell proliferation due to alterations in TR8 receptor expression.

An additional aspect of the invention is related to a method for treating an individual in need of an increased level of a TR8 receptor activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of isolated TR8 polypeptides of the invention or an agonist thereof.

A still further aspect of the invention is related to a method for treating an individual in need of a decreased level of a TR8 receptor activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of a TR8 receptor antagonist.

The invention additionally provides soluble forms of the polypeptides of the present invention. Soluble peptides are defined by amino acid sequences wherein the sequence comprises the polypeptide sequence lacking a transmembrane domain. Such

soluble forms of the TR8 receptors are useful as antagonists of the membrane bound forms of the receptors.

Brief Description of the Figures

FIGS. 1A-C shows the nucleotide sequence (SEQ ID NO:1) and deduced amino acid (SEQ ID NO:2) sequence of a TR8 receptor. Three potential secretory leader sequences have been predicted for the complete polypeptide, of about 21, 23 or 25 amino acid residues, of which the longest, from amino acid 1 to 25 in FIGS. 1A-C, is underlined (amino acid residue -25 to -1 in SEQ ID NO:2). The deduced complete amino acid sequence includes 615 amino acid residues and has a deduced molecular weight of about 65,940 Da. It is further predicted that amino acid residues from about 26 to about 211 in FIGS. 1A-C (amino acid residues 1 to 186 in SEQ ID NO:2) constitute the extracellular domain; from about 212 to about 230 (amino acid residues 187 to 205 in SEQ ID NO:2) the transmembrane domain (underlined); and from about 231 to about 615 (amino acid residues 206 to 590 in SEQ ID NO:2) the intracellular domain.

FIG. 2 shows the regions of similarity between the amino acid sequences of the TR8 receptor protein of FIGS. 1A-C (labeled HDPIK17xxb protein) and a human TNF Receptor II protein (SEQ ID NO:3) which is labeled "TNFR2" (GenBank Accession Number M55994).

FIG. 3 shows an alignment of the amino acid sequences of the TR8 receptor protein of FIGS. 1A-C (labeled HDPIK17xxb) and a human TNF Receptor II protein (SEQ ID NO:3), a human CD40 protein (SEQ ID NO:4; GenBank Accession Number X60592), a human "OX40" surface antigen protein (SEQ ID NO:5, GenBank Accession Number S76792), and a human lymphotoxin-beta ("LTbetaR") (SEQ ID NO:6; GenBank Accession Number L04270).

FIG. 4 shows a structural analysis of the TR8 receptor amino acid sequence of FIGS. 1A-C. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are

shown. In the "Antigenic Index - Jameson-Wolf" graph, amino acid residues 35 to 90, 107 to 210, 236 to 282, 292 to 537 and 556 to 615 in FIGS. 1A-C and FIG. 3 (amino acid residues 10 to 65, 82 to 185, 211 to 257, 267 to 512, and 531 to 590 in SEQ ID NO:2) correspond to the shown highly antigenic regions of the TR8 receptor protein.

FIGS. 5A-B. Schematic diagram of TR8 and its deletions variants.

FIG. 5A. - TR8 and its deletion mutants were fused with a FLAG epitope tag at the N-terminus using the signal sequence in the expression vector pCMVFLAG1 as described in Example 6 under Experimental Procedures. The recited amino acid position corresponds to that depicted in FIGS. 1A-C and FIG. 3. The Roman numerals I, II, and III represent TRAF binding domains within the cytoplasmic domain of TR8.

FIG. 5B. - Amino acid sequence alignment of the TRAF binding domains in various TNFR family members (SEQ ID NOS:18-21) and in human TR8 (SEQ ID NOS:22-24). The recited amino acid position of TR8 corresponds to that depicted in SEQ ID NO:2.

Detailed Description of the Preferred Embodiments

The present invention provides isolated nucleic acid molecules comprising polynucleotides encoding a TR8 polypeptide (FIGS. 1A-C (SEQ ID NO:2)), the amino acid sequence of which was determined by sequencing a cloned cDNA. The TR8 protein shown in FIGS. 1A-C shares sequence homology with the human TNF receptor II (FIG. 2 (SEQ ID NO:3)). On March 13, 1997 a deposit was made at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, and given accession number 97956. The nucleotide sequence shown in FIGS. 1A-C (SEQ ID NO:1) was obtained by sequencing a cDNA clone (Clone ID HDPIK17) containing the same amino acid coding sequences as the clone in ATCC Accession No. 97956. The deposited clone is contained in the pBluescript SK(-) plasmid (Stratagene, La Jolla, CA).

As used herein, "TR8 protein", "TR8 receptor", receptor protein", "TR8", and "TR8 polypeptide" refer to all proteins resulting from the alternate splicing of the genomic DNA sequences encoding proteins having regions of amino acid sequence identity and receptor activity which correspond to the protein shown in FIGS. 1A-C (SEQ ID NO:2). The TR8 protein shown in FIGS. 1A-C is an example of such a receptor protein.

Nucleic Acid Molecules

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Using the information provided herein, such as the nucleotide sequence in FIGS. 1A-C, nucleic acid molecules of the present invention encoding TR8 polypeptides may be obtained using standard cloning and screening procedures, such as those used for cloning cDNAs using mRNA as starting material. Illustrative of the

invention, the nucleic acid molecule described in FIGS. 1A-C (SEQ ID NO:1) was discovered in a cDNA library derived from primary dendritic cells. Other cDNA clones (two) encoding the TR8 polypeptide shown in FIGS. 1A-C were found only in the same cDNA library. One of these exhibited the following changes in the nucleotide and amino acid sequences compared to those shown in FIGS. 1A-C (SEQ ID NOS:1 and 2): an extra CGC codon was inserted after nucleotide 72 resulting in insertion of an additional R residue (after position 3 in SEQ ID NO:2); nucleotide 763 was G instead of A, resulting in the amino acid E instead of F (at position 194 in SEQ ID NO:2); and nucleotide 1583 was G instead of T, resulting in the amino acid S instead of I (at position 487 in SEQ ID NO:2).

The determined nucleotide sequence of the TR8 cDNA of FIGS. 1A-C (SEQ ID NO:1) contains an open reading frame encoding a protein of about 615 amino acid residues, with three potential predicted leader sequences of about 21, 23 or 25 amino acid residues, and a deduced molecular weight of about 65,940 Da. Consistent with this deduced amino acid sequence, expression of a TR8 cDNA clone in a coupled transcription-translation system generated a protein of approximately 70 kDa, which corresponds well with the predicted molecular weight given the limits of accuracy for this determination. The amino acid sequence of the shortest potential predicted mature TR8 receptor is shown in FIGS. 1A-C, from amino acid residue about 26 to residue about 615 (amino acid residues 1 to 590 in SEQ ID NO:2). The TR8 protein shown in FIGS. 1A-C (SEQ ID NO:2) is about 30.4% identical and about 46.9% similar to the human TNF Receptor II protein shown in SEQ ID NO:3 (see FIG. 2) using the computer program "Bestfit" (see below). Using a similar alignment program with somewhat different similarity scoring rules (DNA Star "Megalign") the TR8 protein of FIGS. 1A-C is about 71.5% similar to the same TNF Receptor II protein (SEQ ID NO:3), 61.9% similar to a human CD40 protein (SEQ ID NO:4), 71.7% similar to a human OX40 protein (SEQ ID NO:5) and 72.9% similar to a human lymphotoxin-beta receptor protein (SEQ ID NO:6); see FIG. 3.

As indicated, the present invention also provides mature forms of the TR8 receptor of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species on the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides nucleotide sequences encoding mature TR8 polypeptides having the amino acid sequences encoded by the cDNA clone contained in the host identified as ATCC Deposit Number 97956 and as shown in FIGS. 1A-C (SEQ ID NO:2). By the mature TR8 polypeptide having the amino acid sequences encoded by the cDNA clone contained in the host identified as ATCC Deposit Number 97956 is meant the mature form(s) of the TR8 receptor produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the vector in the deposited host.

Methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the methods of McGeoch (*Virus Res. 3*:271-286 (1985)) and von Heinje (*Nucleic Acids Res. 14*:4683-4690 (1986)) can be used. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. von Heinje, *supra*. However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the predicted amino acid sequence of the complete TR8 polypeptide shown in FIGS. 1A-C (SEQ ID NO:2) was analyzed by a computer program ("PSORT") (K. Nakai and M. Kanehisa, *Genomics 14*:897-911 (1992)),

which is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis by the PSORT program predicted a signal peptide cleavage site between amino acids 23 and 24 in FIGS. 1A-C (-3 and -2 in SEQ ID NO:2). Thereafter, the complete amino acid sequence was further analyzed by visual inspection, applying a simple form of the (-1,-3) rule of von Heine. von Heinje, *supra*. Thus, potential leader sequences for the TR8 protein shown in SEQ ID NO:2 were predicted to consist of amino acid residues -25 to -5, or -25 to -3, or -25 to -1 in SEQ ID NO:2, while the shortest predicted mature TR8 protein corresponding to the longer potential leader consists of amino acid residues 1 to 590 for the TR8 protein shown in SEQ ID NO:2.

As one of ordinary skill would appreciate, however, due to the possibilities of sequencing errors, as well as the variability of cleavage sites for leaders in different known proteins, the TR8 receptor polypeptide encoded by the cDNA of ATCC Deposit Number 97956 comprises about 615 amino acids, but may be anywhere in the range of 605 to 625 amino acids; and the longest predicted leader sequence of this protein is about 25 amino acids, but the actual leader may be anywhere in the range of about 15 to about 35 amino acids.

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified

(partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) shown in FIGS. 1A-C (SEQ ID NO:1); DNA molecules comprising the coding sequence for the mature TR8 receptor shown in FIGS. 1A-C (SEQ ID NO:2) (about the last 590 amino acids); and DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the TR8 receptor protein shown in FIGS. 1A-C (SEQ ID NO:2). Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

In another aspect, the invention provides isolated nucleic acid molecules encoding the TR8 polypeptide having the amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. 97956 on March 13, 1997. In a further embodiment, these nucleic acid molecules will encode a mature polypeptide or the full-length polypeptide lacking the N-terminal methionine. The invention further provides isolated nucleic acid molecules having the nucleotide sequences shown in FIGS. 1A-C (SEQ ID NO:1), the nucleotide sequence of the cDNA contained in the above-described deposited clone; or nucleic acid molecules having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes, and for detecting expression of the TR8 receptor genes of the present invention in human tissue, for instance, by Northern blot analysis.

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNA, the nucleotide sequence

shown in FIGS. 1A-C (SEQ ID NO:1), or complementary strand thereto, is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40, 50, 100, 150, 200, 250, 300, 400, or 500 nt in length. These fragments have numerous uses which include, but are not limited to, diagnostic probes and primers as discussed herein. Of course, larger fragments, such as those of 500-1500 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequences of the deposited cDNA or as shown in FIGS. 1A-C (SEQ ID NO:1). By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNA or the nucleotide sequence as shown in FIGS. 1A-C (SEQ ID NO:1).

Representative examples of TR8 polynucleotide fragments of the invention include, for example, fragments that comprise, or alternatively, consist of a sequence from about nucleotide 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, or 2001-2050, or 2051 to the end of SEQ ID NO:1, or the complementary DNA strand thereto, or the cDNA contained in the deposited clone. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which demonstrates a functional activity. By a polypeptide demonstrating "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a complete or mature TR8 polypeptide. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a TR8 polypeptide for binding) to an anti-TR8 antibody], immunogenicity (ability to generate antibody

which binds to a TR8 polypeptide), and ability to bind to a receptor or ligand for a TR8 polypeptide.

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding one or more TR8 receptor protein domains. In particular embodiments, such nucleic acid fragments include nucleic acid molecules encoding: a polypeptide comprising the TR8 receptor protein of FIGS. 1A-C (SEQ ID NO:2) extracellular domain (predicted to constitute amino acid residues from about 26 to about 211 in FIGS. 1A-C (amino acid residues 1 to 186 in SEQ ID NO:2)); a polypeptide comprising the TR8 receptor transmembrane domain (amino acid residues 212 to 230 in FIGS. 1A-C (amino acid residues 187 to 205 in SEQ ID NO:2)); a polypeptide comprising the TR8 receptor intracellular domain (predicted to constitute amino acid residues from about 231 to about 615 in FIGS. 1A-C (amino acid residues 206 to 590 in SEQ ID NO:2)); and a polypeptide comprising the TR8 receptor protein of FIGS. 1A-C (SEQ ID NO:2) extracellular and intracellular domains with all or part of the transmembrane domain deleted.

As above with the leader sequence, the amino acid residues constituting the extracellular, transmembrane and intracellular domains have been predicted by computer analysis. Thus, as one of ordinary skill would appreciate, the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to about 15 amino acid residues) depending on the criteria used to define each domain.

Preferred nucleic acid fragments of the present invention also include nucleic acid molecules encoding epitope-bearing portions of the TR8 receptor proteins. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 35 to about 90 in FIGS. 1A-C (amino acid residues 10 to 65 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about 107 to about 210 in FIGS. 1A-C (amino acid residues 82 to 185 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about 236 to about 282 in FIGS. 1A-C (amino acid residues 211 to 257 in SEQ ID NO:2); a polypeptide comprising amino acid residues from

about 292 to about 537 in FIGS. 1A-C (amino acid residues 267 to 512 in SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 556 to about 615 in FIGS. 1A-C (amino acid residues 531 to 590 in SEQ ID NO:2). The inventors have determined that the above polypeptide fragments are antigenic regions of the TR8 receptors. Methods for determining other such epitope-bearing portions of the TR8 proteins are described in detail below.

In another aspect, the invention provides isolated nucleic acid molecules comprising polynucleotides which hybridizes, preferably under stringent hybridization conditions, to a portion of the polynucleotide of one of the nucleic acid molecules of the invention described herein, for instance, the cDNA clone contained in ATCC Deposit 97956. By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70, or 80-150 nt, or the entire length of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in FIGS. 1A-C (SEQ ID NO:1).

Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3′ terminal poly(A) tract of a cDNA sequence), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide

would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

As indicated, nucleic acid molecules of the present invention which encode TR8 polypeptides may include, but are not limited to, those encoding the amino acid sequences of the mature polypeptides, by itself; the coding sequence for the mature polypeptides and additional sequences, such as those encoding the potential leader or signal peptide sequences, such as pre-, or pro- or prepro- protein sequences; the coding sequence of the mature polypeptides, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5′ and 3′ sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, the sequence encoding the polypeptides may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz *et al., Proc. Natl. Acad. Sci. USA 86*:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson *et al., Cell 37*:767 (1984). As discussed below, other such fusion proteins include the TR8 receptors fused to IgG-Fc at the N- or C-terminus.

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the TR8 receptors. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given

locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions, which may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the TR8 receptors or portions thereof. Also especially preferred in this regard are conservative substitutions.

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to (a) a nucleotide sequence encoding the TR8 polypeptide having the complete amino acid sequence shown in FIGS. 1A-C (amino acid residues -25 to 590 in SEQ ID NO:2); (b) a nucleotide encoding the complete amino sequence shown in FIGS. 1A-C but lacking the N-terminal methionine (i.e., amino acids -24 to 590 in SEQ ID NO:2); (c) a nucleotide sequence encoding the predicted mature TR8 receptors (full-length polypeptide with any attending leader sequence removed) comprising the amino acid sequence at positions from about 26 to about 615 in FIGS. 1A-C (amino acid residues 1 to 590 in SEQ ID NO:2); (d) a nucleotide sequence encoding the TR8 polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit Number 97956; (e) a nucleotide sequence encoding the mature TR8 receptor having the amino acid sequences encoded by the cDNA clone contained in ATCC Deposit Number 97956; (f) a nucleotide sequence encoding the TR8 receptor extracellular domain; (g) a nucleotide sequence encoding the TR8 receptor transmembrane domain; (h) a nucleotide sequence encoding the TR8 receptor intracellular domain; (i) a nucleotide sequence encoding the TR8 receptor extracellular

and intracellular domains with all or part of the transmembrane domain deleted; and (j) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), or (i).

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a TR8 polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the TR8 receptors. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5′ or 3′ terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The reference (query) sequence may be the entire TR8 nucleotide sequence shown in FIGS. 1A-C (SEQ ID NO:1) or any fragment as described herein.

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in FIGS. 1A-C (SEQ ID NO:1) or to the nucleotides sequence of the deposited cDNA clone encoding that protein can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics 2*: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for

instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. A determination of whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of this embodiment. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score. For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions

occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

The present application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences shown in FIGS. 1A-C (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNA, irrespective of whether they encode a polypeptide having TR8 receptor activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having TR8 receptor activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having TR8 receptor activity include, *inter alia,* (1) isolating a TR8 receptor gene or allelic or splice variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of a TR8 receptor gene, as described in Verma *et al., Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting TR8 receptor mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in FIGS. 1A-C (SEQ ID NO:1), the nucleic acid sequence of the deposited cDNA, or fragments thereof, which do, in fact, encode a polypeptide having TR8 receptor activity. By "a polypeptide having TR8 receptor activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the TR8 receptors of the present invention (either the full-length protein, the splice variants, or, preferably, the mature protein), as measured in a particular biological assay. For example, TR8 receptor activity can be measured by determining the ability of a polypeptide-Fc fusion protein to inhibit lymphocyte proliferation. TR8 receptor activity may also be measured by determining the ability of a polypeptide, such as cognate ligand which is free or expressed on a cell surface, to confer proliferatory activity in intact cells expressing the receptor.

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNA or the nucleic acid sequence shown in FIGS. 1A-C (SEQ ID NO:1), or fragments thereof, will encode polypeptides "having TR8 receptor activity." In fact, since degenerate variants of any of these nucleotide sequences all encode the same polypeptide, in many instances, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having TR8 protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247:*1306-1310 (1990),

wherein the authors indicate that proteins are surprisingly tolerant of amino acid substitutions.

Vectors and Host Cells

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of TR8 polypeptides or fragments thereof using these host cells or host cells that have otherwise been genetically engineered using techniques known in the art to express a polypeptide of the invention.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipID. If the vector is a virus, it may be packaged *in vitro* using an appropriate packaging cell line and then transduced into host cells.

In one embodiment, the DNA of the invention is operatively associated with an appropriate heterologous regulatory element (e.g. promoter or enhancer), such as the phage lambda PL promoter, the *E. coli lac, trp* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters or enhancers will be known to the skilled artisan.

In embodiments in which vectors contain expression constructs, these constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the vector expression constructs will preferably include a translation initiating at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate

heterologous hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pTR840, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Selection of appropriate vectors and promoters for expression in a host cell is a well known procedure and the requisite techniques for expression vector construction, introduction of the vector into the host and expression in the host are routine skills in the art.

The present invention also relates to host cells containing the vector constructs discussed herein, and additionally encompasses host cells containing nucleotide sequences of the invention that are operably associated with one or more heterologous control regions (e.g., promoter and/or enhancer) using techniques known of in the art. The host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. The host strain may be chosen which modulates the expression of the inserted gene sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and modification (e.g., phosphorylation, cleavage) of proteins.

Appropriate cell lines can be chosen to ensure the desired modifications and processing of the foreign protein expressed.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al., Basic Methods In Molecular Biology* (1986).

The polypeptide may be expressed in a modified form, such as a fusion protein (comprising the polypeptide joined via a peptide bond to a heterologous protein sequence (of a different protein)), and may include not only secretion signals, but also additional heterologous functional regions. Such a fusion protein can be made by ligating polynucleotides of the invention and the desired nucleic acid sequence encoding the desired amino acid sequence to each other, by methods known in the art, in the proper reading frame, and expressing the fusion protein product by methods known in the art. Alternatively, such a fusion protein can be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Additionally, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art.

A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in

improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as, human hIL-5 receptor have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett *et al.*, *Journal of Molecular Recognition*, 8:52-58 (1995) and K. Johanson *et al.*, *The Journal of Biological Chemistry*. 270, No. 16:9459-9471 (1995).

TR8 receptors can be recovered and purified from recombinant cell cultures by standard methods which include, but are not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, or alternatively, may be missing the N-terminal methonine, in some cases as a result of host-mediated processes.

TR8 Polypeptides and Fragments

The invention further provides isolated TR8 polypeptides having the amino acid sequence encoded by the deposited cDNAs, or the amino acid sequence in FIGS.

1A-C (SEQ ID NO:2) or a peptide or polypeptide comprising a portion of the above polypeptides.

The polypeptides of this invention may be membrane bound or may be in a soluble circulating form. Soluble peptides are defined by amino acid sequence wherein the sequence comprises the polypeptide sequence lacking the transmembrane domain.

The polypeptides of the present invention may exist as a membrane bound receptor having a transmembrane region and an intra- and extracellular region or they may exist in soluble form wherein the transmembrane domain is lacking. One example of such a form of the TR8 receptor is the TR8 receptor shown in FIGS. 1A-C (SEQ ID NO:2) which contains, in addition to a leader sequence, transmembrane, intracellular and extracellular domains. Thus, this form of the TR8 receptor appears to be localized in the cytoplasmic membrane of cells which express this protein.

It will be recognized in the art that some amino acid sequences of the TR8 receptors can be varied without significant effect to the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. Thus, the invention further includes variations of the TR8 receptors which show substantial TR8 receptor activity or which include regions of TR8 proteins such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated above, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J. U., *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247:*1306-1310 (1990).

Thus, the fragment, derivative or analog of the polypeptide of FIGS. 1A-C (SEQ ID NO:2), or that encoded by the deposited cDNA, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one

in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the TR8 proteins. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al., Clin Exp. Immunol. 2*:331-340 (1967); Robbins *et al., Diabetes 36*:838-845 (1987); Cleland *et al. Crit. Rev. Therapeutic Drug Carrier Systems 10*:307-377 (1993)).

The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade *et al., Nature 361*:266-268 (1993) describes certain mutations resulting in selective binding of TNF-α to only one of the two known types of TNF receptors. Thus, the TR8 receptors of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 1).

TABLE 1

CONSERVATIVE AMINO ACID SUBSTITUTIONS.

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine
Glycine	

In specific embodiments, the number of substitutions, additions or deletions in the amino acid sequence of FIGS. 1A-C and/or any of the polypeptide fragments described herein (e.g., the extracellular domain or intracellular domain) is 75, 70, 60, 50, 40, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 20-10, 5-10, 1-5, 1-3 or 1-2.

Amino acids in the TR8 polypeptides of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* *244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro*, or *in vitro* proliferative activity.

Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al.*, *J. Mol. Biol.* *224*:899-904 (1992) and de Vos *et al.*, *Science* *255*:306-312 (1992)).

The polypeptides of the present invention are preferably provided in an isolated form. By "isolated polypeptide", is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and contained within a recombinant host cell would be considered "isolated" for purposes of the present invention. Also intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host. For example, recombinantly produced versions of the TR8 receptors can be substantially purified by the one-step method described in Smith and Johnson, *Gene* *67*:31-40 (1988).

The polypeptides of the present invention also include the polypeptide encoded by the deposited cDNA including the leader; the polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein); the polypeptide of FIGS. 1A-C (SEQ ID NO:2) including the leader; the polypeptides of FIGS. 1A-C (SEQ ID NO:2) including the leader but minus the N-terminal methionine; the polypeptide of FIGS. 1A-C (SEQ ID NO:2) minus the leader; the extracellular domain, the transmembrane domain, and the intracellular domain of the TR8 receptor shown in FIGS. 1A-C (SEQ ID NO:2); and polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptides described above, and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a TR8 polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of a TR8 receptor. In other

words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in FIGS. 1A-C (SEQ ID NO:2), the amino acid sequence encoded by the deposited cDNA clone, or fragments thereof, can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is

shorter. According to this embodiment, if the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. A determination of whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of this embodiment. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence. For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected.

Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

The polypeptides of the present invention have uses which include, but are not limited to, molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art.

For many proteins, including the extracellular domain of a membrane associated protein or the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. However, even if deletion of one or more amino acids from the N-terminus or C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other TR8 functional activities may still be retained. For example, in many instances, the ability of the shortened protein to induce and/or bind to antibodies which recognize TR8 (preferably antibodies that bind specifically to TR8) will retained irrespective of the size or location of the deletion. Whether a particular polypeptide lacking N-terminal and/or C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

In one embodiment, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the TR8 polypeptide depicted in FIGS. 1A-C (SEQ ID NO:2) or encoded by the cDNA of the deposited clone. Particularly, in one embodiment, N-terminal deletions of the TR8 polypeptide can be described by the general formula m to 590, where m is a number from -24 to 589 corresponding to the position of amino acid identified in SEQ ID NO:2 and preferably, corresponds to one of the N-terminal amino acid residues identified in the N-terminal deletions specified herein. In specific embodiments, N-terminal deletions of the TR8 polypeptide of the invention

comprise, or preferably consist of, amino acid residues: L-2 to A-590; Q-3 to A-590; I-4 to A-590; A-5 to A-590; P-6 to A-590; P-7 to A-590; C-8 to A-590; T-9 to A-590;S-10 to A-590; E-11 to A-590; K-12 to A-590; H-13 to A-590; Y-14 to A-590; E-15 to A-590; H-16 to A-590; L-17 to A-590; G-18 to A-590; R-19 to A-590; C-20 to A-590; C-21 to A-590; N-22 to A-590;K-23 to A-590; C-24 to A-590; E-25 to A-590; P-26 to A-590; G-27 to A-590; K-28 to A-590; Y-29 to A-590; M-30 to A-590; S-31 to A-590; S-32 to A-590; K-33 to A-590; C-34 to A-590; T-35 to A-590;T-36 to A-590; T-37 to A-590; S-38 to A-590; D-39 to A-590; S-40 to A-590; V-41 to A-590; C-42 to A-590; L-43 to A-590; P-44 to A-590;C-45 to A-590; G-46 to A-590; P-47 to A-590; D-48 to A-590; E-49 to A-590; Y-50 to A-590; L-51 to A-590; D-52 to A-590; S-53 to A-590; W-54 to A-590; N-55 to A-590; E-56 to A-590; E-57 to A-590; D-58 to A-590; K-59 to A-590; C-60 to A-590; L-61 to A-590;L-62 to A-590; H-63 to A-590; K-64 to A-590; V-65 to A-590; C-66 to A-590; D-67 to A-590; T-68 to A-590; G-69 to A-590; K-70 to A-590; A-71 to A-590; L-72 to A-590; V-73 to A-590; A-74 to A-590;V-75 to A-590; V-76 to A-590; A-77 to A-590; G-78 to A-590; N-79 to A-590; S-80 to A-590; T-81 to A-590; T-82 to A-590; P-83 to A-590; R-84 to A-590; R-85 to A-590; C-86 to A-590; A-87 to A-590;C-88 to A-590; T-89 to A-590; A-90 to A-590; G-91 to A-590; Y-92 to A-590; H-93 to A-590; W-94 to A-590; S-95 to A-590; Q-96 to A-590; D-97 to A-590; C-98 to A-590; E-99 to A-590; C-100 to A-590; C-101 to A-590; R-102 to A-590; R-103 to A-590; N-104 to A-590; T-105 to A-590; E-106 to A-590; C-107 to A-590; A-108 to A-590; P-109 to A-590; G-110 to A-590; L-111 to A-590; G-112 to A-590; A-113 to A-590; Q-114 to A-590; H-115 to A-590; P-116 to A-590; L-117 to A-590; Q-118 to A-590; L-119 to A-590; N-120 to A-590; K-121 to A-590; D-122 to A-590; T-123 to A-590; V-124 to A-590; C-125 to A-590; K-126 to A-590; P-127 to A-590; C-128 to A-590; L-129 to A-590; A-130 to A-590; G-131 to A-590; Y-132 to A-590; F-133 to A-590; S-134 to A-590; D-135 to A-590; A-136 to A-590; F-137 to A-590; S-138 to A-590; S-139 to A-590; T-140 to A-590; D-141 to A-590; K-142 to A-590; C-143 to A-590; R-144 to A-590; P-145 to A-590; W-146 to A-590; T-147 to A-590; N-148 to A-590; C-149 to A-590; T-

150 to A-590; F-151 to A-590; L-152 to A-590; G-153 to A-590; K-154 to A-590; R-155 to A-590; V-156 to A-590; E-157 to A-590; H-158 to A-590; H-159 to A-590; G-160 to A-590; T-161 to A-590; E-162 to A-590; K-163 to A-590; S-164 to A-590; D-165 to A-590; V-166 to A-590; V-167 to A-590; C-168 to A-590; S-169 to A-590; S-170 to A-590; S-171 to A-590; L-172 to A-590; P-173 to A-590; A-174 to A-590; R-175 to A-590; K-176 to A-590; P-177 to A-590; P-178 to A-590; N-179 to A-590; E-180 to A-590; P-181 to A-590; H-182 to A-590; V-183 to A-590; Y-184 to A-590; L-185 to A-590; P-186 to A-590; G-187 to A-590; L-188 to A-590; I-189 to A-590; I-190 to A-590; L-191 to A-590; L-192 to A-590; L-193 to A-590; F-194 to A-590; A-195 to A-590; S-196 to A-590; V-197 to A-590; A-198 to A-590; L-199 to A-590; V-200 to A-590; A-201 to A-590; A-202 to A-590; I-203 to A-590; I-204 to A-590; F-205 to A-590; G-206 to A-590; V-207 to A-590; C-208 to A-590; Y-209 to A-590; R-210 to A-590; K-211 to A-590; K-212 to A-590; G-213 to A-590; K-214 to A-590; A-215 to A-590; L-216 to A-590; T-217 to A-590; A-218 to A-590; N-219 to A-590; L-220 to A-590; W-221 to A-590; H-222 to A-590; W-223 to A-590; I-224 to A-590; N-225 to A-590; E-226 to A-590; A-227 to A-590; C-228 to A-590; G-229 to A-590; R-230 to A-590; L-231 to A-590; S-232 to A-590; G-233 to A-590; D-234 to A-590; K-235 to A-590; E-236 to A-590; S-237 to A-590; S-238 to A-590; G-239 to A-590; D-240 to A-590; S-241 to A-590; C-242 to A-590; V-243 to A-590; S-244 to A-590; T-245 to A-590; H-246 to A-590; T-247 to A-590; A-248 to A-590; N-249 to A-590; F-250 to A-590; G-251 to A-590; Q-252 to A-590; Q-253 to A-590; G-254 to A-590; A-255 to A-590; C-256 to A-590; E-257 to A-590; G-258 to A-590; V-259 to A-590; L-260 to A-590; L-261 to A-590; L-262 to A-590; T-263 to A-590; L-264 to A-590; E-265 to A-590; E-266 to A-590; K-267 to A-590; T-268 to A-590; F-269 to A-590; P-270 to A-590; E-271 to A-590; D-272 to A-590; M-273 to A-590; C-274 to A-590; Y-275 to A-590; P-276 to A-590; D-277 to A-590; Q-278 to A-590; G-279 to A-590; G-280 to A-590; V-281 to A-590; C-282 to A-590; Q-283 to A-590; G-284 to A-590; T-285 to A-590; C-286 to A-590; V-287 to A-590; G-288 to A-590; G-289 to A-590; G-290 to A-590; P-291 to A-590; Y-292 to A-590; A-293 to A-590; Q-294 to

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K-439 to A-590; R-440 to A-590; G-441 to A-590; P-442 to A-590; L-443 to A-590; P-444 to A-590; Q-445 to A-590; C-446 to A-590; A-447 to A-590; Y-448 to A-590; G-449 to A-590; M-450 to A-590; G-451 to A-590; L-452 to A-590; P-453 to A-590; P-454 to A-590; E-455 to A-590; E-456 to A-590; E-457 to A-590; A-458 to A-590; S-459 to A-590; R-460 to A-590; T-461 to A-590; E-462 to A-590; A-463 to A-590; R-464 to A-590; D-465 to A-590; Q-466 to A-590; P-467 to A-590; E-468 to A-590; D-469 to A-590; G-470 to A-590; A-471 to A-590; D-472 to A-590; G-473 to A-590; R-474 to A-590; L-475 to A-590; P-476 to A-590; S-477 to A-590; S-478 to A-590; A-479 to A-590; R-480 to A-590; A-481 to A-590; G-482 to A-590; A-483 to A-590; G-484 to A-590; S-485 to A-590; G-486 to A-590; I-487 to A-590; S-488 to A-590; P-489 to A-590; G-490 to A-590; G-491 to A-590; Q-492 to A-590; S-493 to A-590; P-494 to A-590; A-495 to A-590; S-496 to A-590; G-497 to A-590; N-498 to A-590; V-499 to A-590; T-500 to A-590; G-501 to A-590; N-502 to A-590; S-503 to A-590; N-504 to A-590; S-505 to A-590; T-506 to A-590; F-507 to A-590; I-508 to A-590; S-509 to A-590; S-510 to A-590; G-511 to A-590; Q-512 to A-590; V-513 to A-590; M-514 to A-590; N-515 to A-590; F-516 to A-590; K-517 to A-590; G-518 to A-590; D-519 to A-590; I-520 to A-590; I-521 to A-590; V-522 to A-590; V-523 to A-590; Y-524 to A-590; V-525 to A-590; S-526 to A-590; Q-527 to A-590; T-528 to A-590; S-529 to A-590; Q-530 to A-590; E-531 to A-590; G-532 to A-590; A-533 to A-590; A-534 to A-590; A-535 to A-590; A-536 to A-590; A-537 to A-590; E-538 to A-590; P-539 to A-590; M-540 to A-590; G-541 to A-590; R-542 to A-590; P-543 to A-590; V-544 to A-590; Q-545 to A-590; E-546 to A-590; E-547 to A-590; T-548 to A-590; L-549 to A-590; A-550 to A-590; R-551 to A-590; R-552 to A-590; D-553 to A-590; S-554 to A-590; F-555 to A-590; A-556 to A-590; G-557 to A-590; N-558 to A-590; G-559 to A-590; P-560 to A-590; R-561 to A-590; F-562 to A-590; P-563 to A-590; D-564 to A-590; P-565 to A-590; C-566 to A-590; G-567 to A-590; G-568 to A-590; P-569 to A-590; E-570 to A-590; G-571 to A-590; L-572 to A-590; R-573 to A-590; E-574 to A-590; P-575 to A-590; E-576 to A-590; K-577 to A-590; A-578 to A-590; S-579 to A-590; R-580 to A-590; P-581 to A-590; V-582 to A-590;

Q-583 to A-590; E-584 to A-590; Q-585 to A-590; of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In another embodiment, N-terminal deletions of the TR8 polypeptide can be described by the general formula m to 184 where m is a number from -24 to 183 corresponding to the amino acid sequence identified in SEQ ID NO:2. In specific embodiments, N terminal deletions of the TR8 of the invention comprise, or preferably, consist of, amino acids residues: L-2 to Y-184; Q-3 to Y-184; I-4 to Y-184; A-5 to Y-184; P-6 to Y-184; P-7 to Y-184; C-8 to Y-184;T-9 to Y-184; S-10 to Y-184; E-11 to Y-184; K-12 to Y-184; H-13 to Y-184; Y-14 to Y-184; E-15 to Y-184; H-16 to Y-184; L-17 to Y-184; G-18 to Y-184; R-19 to Y-184; C-20 to Y-184; C-21 to Y-184; N-22 to Y-184; K-23 to Y-184; C-24 to Y-184; E-25 to Y-184; P-26 to Y-184; G-27 to Y-184; K-28 to Y-184; Y-29 to Y-184; M-30 to Y-184; S-31 to Y-184; S-32 to Y-184; K-33 to Y-184; C-34 to Y-184; T-35 to Y-184; T-36 to Y-184; T-37 to Y-184; S-38 to Y-184; D-39 to Y-184; S-40 to Y-184; V-41 to Y-184; C-42 to Y-184; L-43 to Y-184; P-44 to Y-184; C-45 to Y-184; G-46 to Y-184; P-47 to Y-184; D-48 to Y-184; E-49 to Y-184; Y-50 to Y-184; L-51 to Y-184; D-52 to Y-184; S-53 to Y-184; W-54 to Y-184; N-55 to Y-184; E-56 to Y-184; E-57 to Y-184; D-58 to Y-184; K-59 to Y-184; C-60 to Y-184; L-61 to Y-184; L-62 to Y-184; H-63 to Y-184; K-64 to Y-184; V-65 to Y-184; C-66 to Y-184; D-67 to Y-184; T-68 to Y-184; G-69 to Y-184; K-70 to Y-184; A-71 to Y-184; L-72 to Y-184; V-73 to Y-184; A-74 to Y-184; V-75 to Y-184; V-76 to Y-184; A-77 to Y-184; G-78 to Y-184; N-79 to Y-184; S-80 to Y-184; T-81 to Y-184; T-82 to Y-184; P-83 to Y-184; R-84 to Y-184; R-85 to Y-184; C-86 to Y-184; A-87 to Y-184; C-88 to Y-184; T-89 to Y-184; A-90 to Y-184; G-91 to Y-184; Y-92 to Y-184; H-93 to Y-184; W-94 to Y-184; S-95 to Y-184; Q-96 to Y-184; D-97 to Y-184; C-98 to Y-184; E-99 to Y-184; C-100 to Y-184; C-101 to Y-184; R-102 to Y-184; R-103 to Y-184; N-104 to Y-184; T-105 to Y-184; E-106 to Y-184; C-107 to Y-184; A-108 to Y-184; P-109 to Y-184; G-110 to Y-184; L-111 to Y-184; G-112 to Y-184; A-113 to Y-184; Q-114 to Y-184; H-115 to Y-184; P-116 to Y-184; L-117 to Y-184; Q-118 to Y-184; L-119 to Y-184; N-120 to Y-184; K-121 to

Y-184; D-122 to Y-184; T-123 to Y-184;V-124 to Y-184; C-125 to Y-184; K-126 to Y-184; P-127 to Y-184; C-128 to Y-184; L-129 to Y-184; A-130 to Y-184; G-131 to Y-184; Y-132 to Y-184; F-133 to Y-184; S-134 to Y-184; D-135 to Y-184; A-136 to Y-184; F-137 to Y-184; S-138 to Y-184; S-139 to Y-184; T-140 to Y-184; D-141 to Y-184; K-142 to Y-184;C-143 to Y-184; R-144 to Y-184; P-145 to Y-184; W-146 to Y-184; T-147 to Y-184; N-148 to Y-184; C-149 to Y-184; T-150 to Y-184; F-151 to Y-184; L-152 to Y-184; G-153 to Y-184;K-154 to Y-184; R-155 to Y-184; V-156 to Y-184; E-157 to Y-184; H-158 to Y-184; H-159 to Y-184; G-160 to Y-184; T-161 to Y-184; E-162 to Y-184; K-163 to Y-184; S-164 to Y-184;D-165 to Y-184; V-166 to Y-184; V-167 to Y-184; C-168 to Y-184; S-169 to Y-184; S-170 to Y-184; S-171 to Y-184; L-172 to Y-184; P-173 to Y-184; A-174 to Y-184; R-175 to Y-184; K-176 to Y-184; P-177 to Y-184; P-178 to Y-184; N-179 to Y-184; of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Further embodiments of the invention are directed to C-terminal deletions of the TR8 polypeptide described by the general formula 1 to n, where n is a number from 2-589 corresponding to the position of amino acid residue identified in SEQ ID NO:2 and preferably, corresponds to one of the C-terminal amino acid residues identified in the C-terminal deletions specified herein. In specific embodiments, C terminal deletions of the TR8 polypeptide of the invention comprise or preferably, consist of, amino acid residues: A-1 to K-589; A-1 to A-588; A-1 to G-587; A-1 to G-586; A-1 to Q-585; A-1 to E-584; A-1 to Q-583; A-1 to V-582; A-1 to P-581; A-1 to R-580; A-1 to S-579; A-1 to A-578; A-1 to K-577; A-1 to E-576; A-1 to P-575; A-1 to E-574; A-1 to R-573; A-1 to L-572; A-1 to G-571; A-1 to E-570; A-1 to P-569; A-1 to G-568; A-1 to G-567; A-1 to C-566; A-1 to P-565; A-1 to D-564; A-1 to P-563; A-1 to F-562; A-1 to R-561; A-1 to P-560; A-1 to G-559; A-1 to N-558; A-1 to G-557; A-1 to A-556; A-1 to F-555; A-1 to S-554; A-1 to D-553; A-1 to R-552; A-1 to R-551; A-1 to A-550; A-1 to L-549; A-1 to T-548; A-1 to E-547; A-1 to E-546; A-1 to Q-545; A-1 to V-544; A-1 to P-543; A-1 to R-542; A-1 to G-541; A-1 to M-540; A-1 to P-539; A-1 to E-538; A-1 to A-537; A-1 to A-536; A-1 to A-535; A-1 to A-

534; A-1 to A-533; A-1 to G-532; A-1 to E-531; A-1 to Q-530; A-1 to S-529; A-1 to T-528; A-1 to Q-527; A-1 to S-526; A-1 to V-525; A-1 to Y-524; A-1 to V-523; A-1 to V-522; A-1 to I-521; A-1 to I-520; A-1 to D-519; A-1 to G-518; A-1 to K-517; A-1 to F-516; A-1 to N-515; A-1 to M-514; A-1 to V-513; A-1 to -512; A-1 to G-511; A-1 to S-510; A-1 to S-509; A-1 to I-508; A-1 to F-507; A-1 to T-506; A-1 to S-505; A-1 to N-504; A-1 to S-503; A-1 to N-502; A-1 to G-501; A-1 to T-500; A-1 to V-499; A-1 to N-498; A-1 to G-497; A-1 to S-496; A-1 to A-495; A-1 to P-494; A-1 to S-493; A-1 to Q-492; A-1 to G-491; A-1 to G-490; A-1 to P-489; A-1 to S-488; A-1 to I-487; A-1 to G-486; A-1 to S-485; A-1 to G-484; A-1 to A-483; A-1 to G-482; A-1 to A-481; A-1 to R-480; A-to 1 A-479; A-1 to S-478; A-1 to S-477; A-1 to P-476; A-1 to L-475; A-1 to R-474; A-1 to G-473; A-1 to D-472; A-1 to A-471; A-1 to G-470; A-1 to D-469; A-1 to E-468; A-1 to P-467; A-1 to Q-466; A-to 1 D-465; A-1 to R-464; A-1 to A-463; A-1 to E-462; A-1 to T-461; A-1 to R-460; A-1 to S-459; A-1 to A-458; A-1 to E-457; A-1 to E-456; A-1 to E-455; A-1 to P-454; A-1 to P-453; A-1 to L-452; A-to 1 G-451; A-1 to M-450; A-1 to G-449; A-1 to Y-448; A-1 to A-447; A-1 to C-446; A-1 to Q-445; A-1 to P-444; A-1 to L-443; A-1 to P-442; A-1 to G-441; A-1 to R-440; A-1 to K-439; A-1 to P-438; A-to 1 S-437; A-1 to G-436; A-1 to V-435; A-1 to L-434; A-1 to P-433; A-1 to E-432; A-1 to C-431; A-1 to D-430; A-1 to E-429; A-1 to G-428; A-1 to P-427; A-1 to P-426; A-1 to N-425; A-1 to R-424; A-to 1 C-423; A-1 to G-422; A-1 to T-421; A-1 to C-420; A-1 to V-419; A-1 to D-418; A-1 to A-417; A-1 to W-416; A-1 to N-415; A-1 to P-414; A-1 to S-413; A-1 to P-412; A-1 to S-411; A-1 to A-410; A-to 1 A-409; A-1 to W-408; A-1 to H-407; A-1 to P-406; A-1 to C-405; A-1 to H-404; A-1 to G-403; A-1 to S-402; A-1 to D-401; A-1 to V-400; A-1 to E-399; A-1 to K-398; A-1 to Q-397; A-1 to L-396; A-to 1 Y-395; A-1 to N-394; A-1 to E-393; A-1 to S-392; A-1 to S-391; A-1 to M-390; A-1 to P-389; A-1 to T-388; A-1 to W-387; A-1 to D-386; A-1 to T-385; A-1 to R-384; A-1 to C-383; A-1 to L-382; A-to 1 P-381; A-1 to E-380; A-1 to T-379; A-1 to C-378; A-1 to N-377; A-1 to C-376; A-1 to S-375; A-1 to E-374; A-1 to S-373; A-1 to G-372; A-1 to V-371; A-1 to T-370; A-1 to S-369; A-1 to Q-368; A-to 1 T-367; A-1 to G-

366; A-1 to T-365; A-1 to F-364; A-1 to C-363; A-1 to Q-362; A-1 to S-361; A-1 to L-360; A-1 to S-359; A-1 to D-358; A-1 to N-357; A-1 to E-356; A-1 to G-355; A-1 to V-354; A-to 1 E-353; A-1 to L-352; A-1 to P-351; A-1 to E-350; A-1 to S-349; A-1 to F-348; A-1 to P-347; A-1 to P-346; A-1 to T-345; A-1 to S-344; A-1 to K-343; A-1 to S-342; A-1 to G-341; A-1 to P-340; A-1to E-339; A-1 to T-338; A-1 to L-337; A-1 to F-336; A-1 to L-335; A-1 to L-334; A-1 to Q-333; A-1 to D-332; A-1 to T-331; A-1 to P-330; A-1 to Q-329; A-1 to S-328; A-1 to P-327; A-1 to R-326; A-1to D-325; A-1 to M-324; A-1 to Y-323; A-1 to E-322; A-1 to D-321; A-1 to E-320; A-1 to T-319; A-1 to P-318; A-1 to M-317; A-1 to Q-316; A-1 to R-315; A-1 to F-314; A-1 to S-313; A-1 to D-312; A-to 1 E-311; A-1 to E-310; A-1 to I-309; A-1 to E-308; A-1 to T-307; A-to 1 K-306; A-1 to S-305; A-1 to V-304; A-1 to L-303; A-1 to S-302; A-1 to L-301; A-1 to M-300; A-1 to R-299; A-1 to A-298; A-1 to D-297; A-1 to E-296; A-1 to G-295; A-1 to Q-294; A-1 to A-293; A-to 1 Y-292; A-1 to P-291; A-1 to G-290; A-1 to G-289; A-1 to G-288; A-1 to V-287; A-1 to C-286; A-1 to T-285; A-1 to G-284; A-1 to Q-283; A-1 to C-282; A-1 to V-281; A-1 to G-280; A-1 to G-279; A-to 1 Q-278; A-1 to D-277; A-1 to P-276; A-1 to Y-275; A-1 to C-274; A-1 to M-273; A-1 to D-272; A-1 to E-271; A-1 to P-270; A-1 to F-269; A-1 to T-268; A-1 to K-267; A-1 to E-266; A-1 to E-265; A-1to L-264; A-1 to T-263; A-1 to L-262; A-1 to L-261; A-1 to L-260; A-1 to V-259; A-1 to G-258; A-1 to E-257; A-1 to C-256; A-1 to A-255; A-1 to G-254; A-1 to Q-253; A-1 to Q-252; A-1 to G-251; A-to 1 F-250; A-1 to N-249; A-1 to A-248; A-1 to T-247; A-1 to H-246; A-1 to T-245; A-1 to S-244; A-1 to V-243; A-1 to C-242; A-1 to S-241; A-1 to D-240; A-1 to G-239; A-1 to S-238; A-1 to S-237; A-to 1 E-236; A-1 to K-235; A-1 to D-234; A-1 to G-233; A-1 to S-232; A-1 to L-231; A-1 to R-230; A-1 to G-229; A-1 to C-228; A-1 to A-227; A-1 to E-226; A-1 to N-225; A-1 to I-224; A-1 to W-223; A-to 1 H-222; A-1 to W-221; A-1 to L-220; A-1 to N-219; A-1 to A-218; A-1 to T-217; A-1 to L-216; A-1 to A-215; A-1 to K-214; A-1 to G-213; A-1 to K-212; A-1 to K-211; A-1 to R-210; A-1 to Y-209; A-to 1 C-208; A-1 to V-207; A-1 to G-206; A-1 to F-205; A-1 to I-204; A-1 to I-203; A-1 to A-202; A-1 to A-201; A-1 to V-200; A-1 to L-

199; A-1 to A-198; A-1 to V-197; A-1 to S-196; A-1 to A-195; A-to 1 F-194; A-1 to L-193; A-1 to L-192; A-1 to L-191; A-1 to I-190; A-to 1 I-189; A-1 to L-188; A-1 to G-187; A-1 to P-186; A-1 to L-185; A-1 to Y-184; A-1 to V-183; A-1 to H-182; A-1 to P-181; A-1 to E-180; A-1 to N-179; A-1 to P-178; A-1 to P-177; A-1 to K-176; A-to 1 R-175; A-1 to A-174; A-1 to P-173; A-1 to L-172; A-1 to S-171; A-1 to S-170; A-1 to S-169; A-1 to C-168; A-1 to V-167; A-1 to V-166; A-1 to D-165; A-1 to S-164; A-1 to K-163; A-1 to E-162; A-to 1 T-161; A-1 to G-160; A-1 to H-159; A-1 to H-158; A-1 to E-157; A-1 to V-156; A-1 to R-155; A-1 to K-154; A-1 to G-153; A-1 to L-152; A-1 to F-151; A-1 to T-150; A-1 to C-149; A-1 to N-148; A-to 1 T-147; A-1 to W-146; A-1 to P-145; A-1 to R-144; A-1 to C-143; A-1 to K-142; A-1 to D-141; A-1 to T-140; A-1 to S-139; A-1 to S-138; A-1 to F-137; A-1 to A-136; A-1 to D-135; A-1 to S-134; A-1to F-133; A-1 to Y-132; A-1 to G-131; A-1 to A-130; A-1 to L-129; A-1 to C-128; A-1 to P-127; A-1 to K-126; A-1 to C-125; A-1 to V-124; A-1 to T-123; A-1 to D-122; A-1 to K-121; A-1 to N-120; A-to 1 L-119; A-1 to Q-118; A-1 to L-117; A-1 to P-116; A-1 to H-115; A-1 to Q-114; A-1 to A-113; A-1 to G-112; A-1 to L-111; A-1 to G-110; A-1 to P-109; A-1 to A-108; A-1 to C-107; A-1 to E-106; A-to 1 T-105; A-1 to N-104; A-1 to R-103; A-1 to R-102; A-1 to C-101; A-1 to C-100; A-1 to E-99; A-1 to C-98; A-1 to D-97; A-1 to Q-96; A-1 to S-95; A-1 to W-94; A-1 to H-93; A-1 to Y-92; A-1 to G-91; A-1 to A-90; A-1 to T-89; A-1 to C-88; A-1 to A-87; A-1 to C-86; A-to 1 R-85; A-1 to R-84; A-1 to P-83; A-1 to T-82; A-1 to T-81; A-1 to S-80; A-1 to N-79; A-1 to G-78; A-1 to A-77; A-1 to V-76; A-1 to V-75; A-1 to A-74; A-1 to V-73; A-1 to L-72; A-1 to A-71; A-1 to K-70; A-1 to G-69; A-1 to T-68; A-1 to D-67; A-1 to C-66; A-1 to V-65; A-1 to K-64; A-1 to H-63; A-1 to L-62; A-1 to L-61; A-1 to C-60; A-1 to K-59; A-1 to D-58; A-1 to E-57; A-1 to E-56; A-1 to N-55; A-1 to W-54; A-1 to S-53; A-1 to D-52; A-1 to L-51; A-1 to Y-50; A-1 to E-49; A-1 to D-48; A-1 to P-47; A-1 to G-46; A-1 to C-45; A-1 to P-44; A-1 to L-43; A-1 to C-42; A-1 to V-41; A-1 to S-40; A-1 to D-39; A-1 to S-38; A-1 to T-37; A-1 to T-36; A-1 to T-35; A-1 to C-34; A-1 to K-33; A-1 to S-32; A-1 to S-31; A-1 to M-30; A-1 to Y-29; A-1 to K-28; A-1 to G-27; A-1 to P-26; A-1

to E-25; A-1 to C-24; A-1 to K-23; A-1 to N-22; A-1 to C-21; A-1 to C-20; A-1 to R-19; A-1 to G-18; A-1 to L-17; A-1 to H-16; A-1 to E-15; A-1 to Y-14; A-1 to H-13; A-1 to K-12; A-1 to E-11; A-1 to S-10; A-1 to T-9; A-1 to C-8; A-1 to P-7; A-1 to P-6; of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Further embodiments of the invention are directed to polypeptide fragments comprising, or preferably, consisting of, amino acids described by the general formula m to n, where m and n correspond to any one of the amino acid residues specified above for these symbols, respectively.

Polypeptide fragments of the present invention include polypeptides comprising an amino acid sequence contained in SEQ ID NO:2, encoded by the cDNA contained in the deposited clone, or encoded by nucleic acids which hybridize (e.g., under stringent hybridization conditions) to the nucleotide sequence contained in the deposited clone, or shown in FIGS. 1A-C (SEQ ID NO:1) or the complementary strand thereto. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments that comprise or alternatively, consist of from about amino acid residues -25 to 1, 1 to 20, 21 to 40, 41 to 60, 61 to 80, 81 to 100, 102 to 120, 121 to 140, 141 to 160, 161 to 180, 181 to 200, 201 to 220, 221 to 240, 241 to 260, 261 to 280, 281 to 310, 311 to 350, 351 to 400, 401 to 450, 451 to 500, 551 to 600, or 601 to the end of the coding region of SEQ ID NO:2. Moreover, polypeptide fragments can be at least about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

Among the especially preferred fragments of the invention are fragments characterized by structural or functional attributes of TR8. Such fragments include amino acid residues that comprise alpha-helix and alpha-helix forming regions (“alpha-

regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophillic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, surface forming regions, and high antigenic index regions (i.e., having an antigenic index of or equal to greater than 0.7, as identified using the default parameters of the Jameson-Wolf program) of TR8. Certain preferred regions are those set out in FIG. 4 and include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence depicted in FIGS. 1A-C, such preferred regions include; Garnier-Robson predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Kyte-Doolittle predicted hydrophilic and hydrophobic regions; Eisenberg alpha and beta amphipathic regions; Emini surface-forming regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these computer programs. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In specific embodiments, polypeptide fragments of the invention compris, or alternatively consist of, amino acid residues: 20 to 34, 20 to 60, 20 to 66, 20 to 168, 45 to 60, 66 to 86, 86 to 106, 88 to 100, 101 to 125, 128 to 143, 143 to 149, 149 to 168, 41 to 46, 123 to 128, and/or 138 to 150 as depticted in SEQ ID NO:2.

In additional specific embodiments, polypeptide fragments of the invention comprise one or more of the three potential conserved (boxed) TRAF binding domains in TR8 (See, FIG. 5B)

In other embodiments, the fragments or polypeptides of the invention (i.e., those described herein) are not larger than 570, 550, 525, 500, 475, 450, 400, 425, 390, 380, 375, 350, 336, 334, 331, 300, 275, 250, 225, 200, 185, 175, 170, 165, 160, 155, 150, 145, 140, 135, 130, 125, 120, 115, 110, 105, 100, 90, 80, 75, 60, 50, 40, 30, or 25 amino acid residues in length.

In another aspect, the invention provides peptides or polypeptides comprising epitope-bearing portions of the polypeptides of the invention. The epitopes of these

polypeptide portions are an immunogenic or antigenic epitopes of the polypeptides described herein. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al., Proc. Natl. Acad. Sci. USA 81*:3998- 4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R.A. (1983). Antibodies that react with predetermined sites on proteins. *Science 219:*660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al., Cell 37*:767-778 (1984) at 777. Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between at least about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention.

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate TR8 receptor-specific antibodies include: a polypeptide comprising amino acid residues from about 35 to about 90 in FIGS. 1A-C (amino acid residues 10 to 65 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about 107 to about 210 in FIGS. 1A-C (amino acid residues 82 to 185 in SEQ ID NO:2); a

polypeptide comprising amino acid residues from about 236 to about 282 in FIGS. 1A-C (amino acid residues 211 to 257 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about 292 to about 537 in FIGS. 1A-C (amino acid residues 267 to 512 in SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 556 to about 615 in FIGS. 1A-C (amino acid residues 531 to 590 in SEQ ID NO:2). As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the TR8 receptor proteins.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. Houghten, R. A. (1985) General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. USA 82*:5131-5135. This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

As one of skill in the art will appreciate, TR8 polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker *et al., Nature 331*:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric TR8 receptor proteins or protein fragments alone (Fountoulakis *et al., J. Biochem 270*:3958-3964 (1995)).

Detection of Disease States

The TNF-family ligands induce various cellular responses by binding to TNF-family receptors, including the TR8 receptors of the present invention. TNF-β, a potent ligand of the TNF receptor proteins, is known to be involved in a number of

biological processes including lymphocyte development, tumor necrosis, induction of an antiviral state, activation of polymorphonuclear leukocytes, induction of class I major histocompatibility complex antigens on endothelial cells, induction of adhesion molecules on endothelium and growth hormone stimulation (Ruddle and Homer, *Prog. Allergy, 40:*162-182 (1988)). TNF-α, also a ligand of the TNF receptor proteins, has been reported to have a role in the rapid necrosis of tumors, immunostimulation, autoimmune disease, graft rejection, producing an anti-viral response, septic shock, cerebral malaria, cytotoxicity, protection against deleterious effects of ionizing radiation produced during a course of chemotherapy, such as denaturation of enzymes, lipid peroxidation and DNA damage (Nata *et al, J. Immunol. 136(7):*2483 (1987); Porter, *Tibtech 9*:158-162 (1991)), growth regulation, vascular endothelium effects and metabolic effects. TNF-α also triggers endothelial cells to secrete various factors, including PAI-1, IL-1, GM-CSF and IL-6 to promote cell proliferation. In addition, TNF-α up-regulates various cell adhesion molecules such as E-Selectin, ICAM-1 and VCAM-1. TNF-α and the Fas ligand have also been shown to induce programmed cell death.

Cells which express the TR8 polypeptides and are believed to have a potent cellular response to TR8 receptor ligands include dendritic cells. In addition, Northern blots revealed an approximately 4 kb mRNA observed most abundantly in colon, to a lesser extent in small intestine, lymph node and pancreas, barely detectable in spleen, fetal liver, lung prostate, thymus, testis and ovary, which was not observed in peripheral blood leukocytes, bone marrow, heart, brain, liver, skeletal muscle or kidney. By "a cellular response to a TNF-family ligand" is intended any genotypic, phenotypic, and/or morphologic change to a cell, cell line, tissue, tissue culture or patient that is induced by a TNF-family ligand. As indicated, such cellular responses include not only normal physiological responses to TNF-family ligands, but also diseases associated with increased cell proliferation or the inhibition of increased cell proliferation, such as by the inhibition of apoptosis. Apoptosis-programmed cell

death-is a physiological mechanism involved in the deletion of peripheral T lymphocytes of the immune system, and its dysregulation can lead to a number of different pathogenic processes (Ameisen, J.C., *AIDS 8*:1197-1213 (1994); Krammer *et al., Curr. Opin. Immunol. 6*:279-289 (1994)).

It is believed that certain tissues in mammals with specific disease states associated with aberrant cell survival express significantly altered levels of the TR8 receptor protein and mRNA encoding the TR8 receptor protein when compared to a corresponding "standard" mammal, i.e., a mammal of the same species not having the disease state. Further, since some forms of this protein are secreted, it is believed that enhanced levels of the TR8 receptor protein can be detected in certain body fluids (e.g., sera, plasma, urine, and spinal fluid) from mammals with the disease state when compared to sera from mammals of the same species not having the disease state. Thus, the invention provides a diagnostic method useful during diagnosis of disease states, which involves assaying the expression level of the gene encoding the TR8 receptor protein in mammalian cells or body fluid and comparing the gene expression level with a standard TR8 receptor gene expression level, whereby an increase or decrease in the gene expression level over the standard is indicative of certain disease states associated with aberrant cell survival.

Where diagnosis of a disease state involving the TR8 receptors of the present invention has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting significantly aberrant TR8 receptor gene expression will experience a worse clinical outcome relative to patients expressing the gene at a lower level.

By "assaying the expression level of the gene encoding the TR8 receptor protein" is intended qualitatively or quantitatively measuring or estimating the level of the TR8 receptor protein or the level of the mRNA encoding the TR8 receptor protein in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the TR8 receptor protein level or mRNA level in a second biological sample).

Preferably, the TR8 receptor protein level or mRNA level in the first biological sample is measured or estimated and compared to a standard TR8 receptor protein level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disease state. As will be appreciated in the art, once a standard TR8 receptor protein level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source which contains TR8 receptor protein or mRNA. Biological samples include mammalian body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain secreted mature TR8 receptor protein, and thymus, prostate, heart, placenta, muscle, liver, spleen, lung, kidney and other tissues. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

Diseases associated with increased cell survival, or the inhibition of apoptosis, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors); autoimmune disorders (such as systemic lupus erythematosus and immune-related glomerulonephritis rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), information graft v. host disease, acute graft rejection, and chronic graft rejection. Diseases associated with decreased cell survival, or increased apoptosis, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration); myelodysplastic syndromes (such as aplastic anemia), ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Assays available to detect levels of soluble receptors are well known to those of skill in the art, for example, radioimmunoassays, competitive-binding assays, Western blot analysis, and preferably an ELISA assay may be employed.

TR8 receptor-protein specific antibodies can be raised against the intact TR8 receptor protein or an antigenic polypeptide fragment thereof, which may presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (mAb) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab Fab (ab') fragments) which are capable of specifically binding to TR8 receptor protein. Fab and F(ab') fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl *et al., J. Nucl. Med. 24*:316-325 (1983)). Thus, these fragments are preferred.

The antibodies of the present invention may be prepared by any of a variety of methods using TR8 receptor immunogens of the present invention. Such TR8 receptor immunogens include the TR8 receptor protein shown in FIGS. 1A-C (SEQ ID NO:2) (which may or may not include a leader sequence) and polypeptide fragments of the receptor comprising the ligand binding, extracellular, transmembrane, the intracellular domains of the TR8 receptors, or any combination thereof. For example, cells expressing the TR8 receptor protein or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of TR8 receptor protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or TR8 receptor protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology (Kohler *et al., Nature 256*:495 (1975); Kohler *et al., Eur. J. Immunol. 6*:511 (1976); Kohler *et al., Eur. J. Immunol. 6*:292 (1976); Hammerling *et al.*, In: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., (1981) pp. 563-681). In general, such procedures

involve immunizing an animal (preferably a mouse) with a TR8 receptor protein antigen or, more preferably, with a TR8 receptor protein-expressing cell. Suitable cells can be recognized by their capacity to bind anti-TR8 receptor protein antibody. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56 C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin. The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP_2O), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands *ét al.*, (*Gastroenterology 80*:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the TR8 receptor protein antigen.

Antibodies of the invention can be used in methods known in the art relating to the localization and activity of the polypeptide sequences of the invention, e.g., for imaging these polypeptides, measuring levels thereof in appropriate physiological samples, etc. The antibodies also have use in immunoassays and in therapeutics as agonists and antagonists of TR8.

Agonists and Antagonists of TR8 Receptor Function

In one aspect, the present invention is directed to a method for inhibiting an activity of TR8 induced by a TNF-family ligand (e.g., cell proliferation, hematopoietic development, osteoclast differentiation, and survival of dendritic cells), which involves administering to a cell which expresses a TR8 polypeptide, an effective amount of a TR8 receptor ligand, analog or an antagonist capable of decreasing TR8, receptor mediated signaling. Preferably, TR8 receptor mediated signaling is decreased to treat a disease wherein increased cell proliferation is exhibited. An antagonist can include soluble forms of the TR8 receptors and antibodies directed against the TR8 polypeptides which block TR8 receptor mediated signaling. Preferably, TR8 receptor mediated signaling is decreased to treat a disease, to decrease survival of cells, e.g., dendritic cells, or to delay or prevent bone formation (e.g., via osteoclast differentiation).

In a further aspect, the present invention is directed to a method for increasing cell proliferation induced by a TNF-family ligand, which involves administering to a cell which expresses a TR8 polypeptide an effective amount of an agonist capable of increasing TR8 receptor mediated signaling. Preferably, TR8 receptor mediated signaling is increased to treat a disease wherein decreased cell proliferation is exhibited wherein increased survival of cells (e.g., dendritic cells) is desired, or to stimulate bone formation (e.g., via osteoclast differentiation). Agonists of the present invention include monoclonal antibodies directed against the TR8 polypeptides which stimulate TR8 receptor mediated signaling. Preferably, TR8 receptor mediated signaling is increased to treat a disease.

By "agonist" is intended naturally occurring and synthetic compounds capable of enhancing cell proliferation, survival, and/or differentiation mediated by TR8 polypeptides. Such agonists include agents which increase expression of TR8 receptors or increase the sensitivity of the expressed receptor. By "antagonist" is intended naturally occurring and synthetic compounds capable of inhibiting TR8

mediated cell proliferation and differentiation. Such antagonists include agents which decrease expression of TR8 receptors or decrease the sensitivity of the expressed receptor. Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit cell proliferation, survival, and differentiation can be determined using art-known TNF-family ligand/receptor cellular response assays, including those described in more detail below.

One such screening technique involves the use of cells which express the receptor (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation, for example, as described in *Science 246*:181-296 (October 1989). For example, compounds may be contacted with a cell which expresses the receptor polypeptide of the present invention and a second messenger response, e.g., signal transduction or pH changes, may be measured to determine whether the potential compound activates or inhibits the receptor.

Another such screening technique involves introducing RNA encoding the receptor into *Xenopus* oocytes to transiently express the receptor. The receptor oocytes may then be contacted with the receptor ligand and a compound to be screened, followed by detection of inhibition or activation of a calcium signal in the case of screening for compounds which are thought to inhibit activation of the receptor.

Another method involves screening for compounds which inhibit activation of the receptor polypeptide of the present invention antagonists by determining inhibition of binding of labeled ligand to cells which have the receptor on the surface thereof. Such a method involves transfecting a eukaryotic cell with DNA encoding the receptor such that the cell expresses the receptor on its surface and contacting the cell with a compound in the presence of a labeled form of a known ligand. The ligand can be labeled, e.g., by radioactivity. The amount of labeled ligand bound to the receptors is measured, e.g., by measuring radioactivity of the receptors. If the compound binds to the receptor as determined by a reduction of labeled ligand which binds to the receptors, the binding of labeled ligand to the receptor is inhibited.

Soluble forms of the polypeptides of the present invention may be utilized in the ligand binding assay described above. These forms of the TR8 receptors are contacted with ligands in the extracellular medium after they are secreted. A determination is then made as to whether the secreted protein will bind to TR8 receptor ligands.

Further screening assays for agonist and antagonist of the present invention are described in Tartaglia and Goeddel, *J. Biol. Chem. 267(7)*:4304-4307(1992).

Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to a TNF-family ligand. The method involves contacting cells which express TR8 polypeptides with a candidate compound and a TNF-family ligand, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of the ligand/receptor signaling pathway and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of the ligand/receptor signaling pathway. By "assaying a cellular response" is intended qualitatively or quantitatively measuring a cellular response to a candidate compound and/or a TNF-family ligand (e.g., determining or estimating an increase or decrease in T cell proliferation or tritiated thymidine labeling). By the invention, a cell expressing a TR8 polypeptide can be contacted with either an endogenous or exogenously administered TNF-family ligand.

In an additional aspect, a thymocyte proliferation assay may be employed to identify both ligands and potential drug candidates. For example, thymus cells are disaggregated from tissue and grown in culture medium. Incorporation of DNA precursors such as ^{3}H-thymidine or 5-bromo-2'-deoxyuridine (BrdU) is monitored as a parameter for DNA synthesis and cellular proliferation. Cells which have incorporated BrdU into DNA can be detected using a monoclonal antibody against BrdU and measured by an enzyme or fluorochrome-conjugated second antibody. The

reaction is quantitated by fluorimetry or by spectrophotometry. Two control wells and an experimental well are set up as above and TNF-β or cognate ligand is added to all wells while soluble receptor polypeptides of the present invention are added individually to the second control wells, with the experimental well containing a compound to be screened. The ability of the compound to be screened to stimulate or inhibit the above interaction may then be quantified.

Agonists according to the present invention include compounds such as, for example, TNF-family ligand peptide fragments, transforming growth factors, and neurotransmitters (such as glutamate, dopamine, *N*-methyl-D-aspartate). Preferred agonists include TR8 polypeptide fragments of the invention and/or polyclonal and monoclonal antibodies raised against TR8 polypeptide, or a fragment thereof. Such agonist antibodies raised against a TNF-family receptor are disclosed in Tartaglia, L.A., *et al., Proc. Natl. Acad. Sci. USA 88*:9292-9296 (1991); and Tartaglia, L.A., and Goeddel, D.V., *J* (7):4304-4307 (1992). See, also, PCT Application WO 94/09137. Further preferred agonists include chemotherapeutic drugs such as, for example, cisplatin, doxorubicin, bleomycin, cytosine arabinodide, nitrogen mustard, methotrexate and vincristine. Others include ethanol and -amyloid peptide. (*Science 267:*1457-1458 (1995)).

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in FIGS. 1A-C, or the complementary strand thereof, and/or to nucleotide sequences contained in the deposited clone. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, J., *Neurochem.* 56:560 (1991). *Oligodeoxynucleotides as Anitsense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, J., *Neurochem.* 56:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Triple helix

formation is discussed in, for instance, Lee et al., *Nucleic Acids Research* 6:3073 (1979); Cooney et al., *Science* 241:456 (1988); and Dervan et al., *Science* 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the TR8 antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the TR8 antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others know in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding TR8, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature* 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci.* U.S.A. 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, et al., *Nature* 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a TR8 gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded TR8 antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid Generally, the larger the hybridizing nucleic acid, the more base mismatches with a TR8 RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, *Science* 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy TR8 mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, *Nature* 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of TR8 (FIGS. 1A-C). Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the TR8 mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. Since ribozymes,

unlike antisense molecules are catalytic, a lower intracellular concentration is required for efficiency.

In other embodiments, antagonists according to the present invention include soluble forms of the TR8 receptors (e.g., fragments of the TR8 receptor shown in FIGS. 1A-C that include the ligand binding domain from the extracellular region of the full length receptor). Such soluble forms of the receptor, which may be naturally occurring or synthetic, antagonize TR8 mediated signaling by competing with the cell surface bound forms of the receptor for binding to TNF-family ligands. Antagonists of the present invention also include antibodies specific for TNF-family ligands and TR8-Fc fusion proteins.

By a "TNF-family ligand" is intended naturally occurring, recombinant, and synthetic ligands that are capable of binding to a member of the TNF receptor family and inducing the ligand/receptor signaling pathway. Members of the TNF ligand family include, but are not limited to, TNF-α, lymphotoxin-α (LT-α, also known as TNF-β), LT-β (found in complex heterotrimer LT-α2-β), FasL, CD40L, CD27L, CD30L, 4-IBBL, OX40L and nerve growth factor (NGF).

TNF-α has been shown to protect mice from infection with herpes simplex virus type 1 (HSV-1). Rossol-Voth *et al., J .Gen. Virol. 72*:143-147 (1991). The mechanism of the protective effect of TNF-α is unknown but appears to involve neither interferons nor NK cell killing. One member of the TNFR family has been shown to mediate HSV-1 entry into cells. Montgomery *et al., Eur. Cytokine Newt. 7*:159 (1996). Further, antibodies specific for the extracellular domain of this TNFR block HSV-1 entry into cells. Thus, TR8 antagonists of the present invention include both TR8 amino acid sequences and antibodies capable of preventing TNFR mediated viral entry into cells. Such sequences and antibodies can function by either competing with cell surface localized TNFR for binding to virus or by directly blocking binding of virus to cell surface receptors.

Antibodies according to the present invention may be prepared by any of a variety of standard methods using TR8 receptor immunogens of the present invention. Such TR8 receptor immunogens include the TR8 receptor protein shown in FIGS. 1A-C (SEQ ID NO:2) (which may or may not include a leader sequence) and polypeptide fragments of the receptor comprising the ligand binding, extracellular, transmembrane, the intracellular domains of the TR8 receptors, or any combination thereof.

Polyclonal and monoclonal antibody agonists or antagonists according to the present invention can be raised according to the methods disclosed in Tartaglia and Goeddel, *J. Biol. Chem.* 267(7):4304-4307(1992)); Tartaglia *et al., Cell 73*:213-216 (1993)), and PCT Application WO 94/09137 and are preferably specific to polypeptides of the invention having the amino acid sequence of SEQ ID NO:2. The term "antibody" (Ab) or "monoclonal antibody" (mAb) as used herein is meant to include intact molecules as well as fragments thereof (such as, for example, Fab and F(ab') fragments) which are capable of binding an antigen. Fab, Fab' and F(ab') fragments lack the Fc fragment intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl *et al., J. Nucl. Med., 24*:316-325 (1983)).

In a preferred method, antibodies according to the present invention are mAbs. Such mAbs can be prepared using hybridoma technology (Kohler and Millstein, *Nature 256*:495-497 (1975) and U.S. Patent No. 4,376,110; Harlow *et al., Antibodies*: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988; *Monoclonal Antibodies and Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, New York, NY, 1980; Campbell, "Monoclonal Antibody Technology," In: *Laboratory Techniques in Biochemistry and Molecular Biology*, Volume 13 (Burdon *et al.*, eds.), Elsevier, Amsterdam (1984)).

Proteins and other compounds which bind the TR8 receptor domains are also candidate agonist and antagonist according to the present invention. Such binding compounds can be "captured" using the yeast two-hybrid system (Fields and Song,

Nature 340:245-246 (1989)). A modified version of the yeast two-hybrid system has been described by Roger Brent and his colleagues (Gyuris *et al., Cell 75*:791-803 (1993); Zervos *et al., Cell 72*:223-232 (1993)). Preferably, the yeast two-hybrid system is used according to the present invention to capture compounds which bind to the ligand binding, extracellular, intracellular, and transmembrane domains of the TR8 receptors. Such compounds are good candidate agonist and antagonist of the present invention.

Using the two-hybrid assay described above, the intracellular domain of the TR8 receptor, or a portion thereof, may be used to identify cellular proteins which interact with the receptor *in vivo*. Such an assay may also be used to identify ligands with potential agonistic or antagonistic activity of TR8 receptor function. This screening assay has previously been used to identify protein which interact with the cytoplasmic domain of the murine TNF-RII and led to the identification of two receptor associated proteins. Rothe, M. *et al., Cell 78*:681 (1994). Such proteins and amino acid sequences which bind to the cytoplasmic domain of the TR8 receptors are good candidate agonist and antagonist of the present invention.

Other screening techniques include the use of cells which express the polypeptide of the present invention (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation, for example, as described in *Science*, *246*:181-296 (1989). In another example, potential agonists or antagonists may be contacted with a cell which expresses the polypeptide of the present invention and a second messenger response, e.g., signal transduction may be measured to determine whether the potential antagonist or agonist is effective.

The TR8 receptor agonists may be employed to stimulate ligand activities, such as inhibition of tumor growth and necrosis of certain transplantable tumors, or alternatively, the survival of certain cell types (e.g., dendritic cells). The agonists may also be employed to stimulate cellular differentiation, for example, T-cells, osteoclasts, fibroblasts and hemopoietic cell differentiation. Agonists to the TR8 receptor may also augment TR8's role in the host's defense against microorganisms and prevent

related diseases (infections such as that from *Listeria monocytogenes*) and Chlamidiae. The agonists may also be employed to protect against the deleterious effects of ionizing radiation produced during a course of radiotherapy, such as denaturation of enzymes, lipid peroxidation, and DNA damage.

Agonists to the receptor polypeptides of the present invention may be used to augment TNF's role in host defenses against microorganisms and prevent related diseases. The agonists may also be employed to protect against the deleterious effects of ionizing radiation produced during a course of radiotherapy, such as denaturation of enzymes, lipid peroxidation, and DNA damage.

The agonists may also be employed to mediate an anti-viral response, to regulate growth, to mediate the immune response and to treat immunodeficiencies related to diseases such as HIV by increasing the rate of lymphocyte proliferation and differentiation.

Agonists to the receptor polypeptides of the present invention may additionally be used to effectuate bone growth (i.e., bone mass). Administration of such agonists can be used to treat bone fractures, defects, and disorders which result in weakened bones such as osteoporosis, osteomalacia, and age-related loss of bone mass. According to the invention, bone growth is enhanced by local and/or systemic administration of a TR8 agonist in an osteogenically effective amount (i.e., an amount which effects the formation and/or development of bone). Additionally, agonists of the invention may optionally be combined with osteogenically effective amounts of other bone growth promoting compounds, including beta-type transforming growth factors ("TGF-βs"); e.g., TGF-β1, 2, 3 and/or bone morphogenic proteins ("BMPs"; e.g., BMP-2,3,4,5, 6, or 7) and osteogenic proteins, and/or parathyroid hormone. BMPs and TGF-βs may be prepared by methods known in the art (see e.g., PCT/US87/01537 and U.S. Patent 4,774,332 which are incorporated herein by reference in their entirety). Alternatively, TGF-βs are available from commercial sources (R&D Systems, Minneapolis, Minn.).

The antagonists to the polypeptides of the present invention may be employed to inhibit ligand activities, such as, for example, stimulation of tumor growth and necrosis of certain transplantable tumors, and promoting the survival of certain cell types (e.g., dendritic cells). The antagonists may also be employed to inhibit cellular differentiation, such as, for example, T-cell, osteoclast, fibroblast, and hemopoietic cell differentiation. Antagonists may also be employed to treat autoimmune diseases, such as, for example, graft versus host rejection and allograft rejection, and T-cell mediated autoimmune diseases such as AIDS. It has been shown that T-cell proliferation is stimulated via a type 2 TNF receptor. Accordingly, antagonizing the receptor may prevent the proliferation of T-cells and treat T-cell mediated autoimmune diseases.

The state of immunodeficiency that defines AIDS is secondary to a decrease in the number and function of $CD4^+$ T-lymphocytes. Recent reports estimate the daily loss of $CD4^+$ T cells to be between 3.5×10^7 and 2×10^9 cells (Wei *et al., Nature 373*:117-122 (1995)). One cause of $CD4^+$ T cell depletion in the setting of HIV infection is believed to be HIV-induced apoptosis. Indeed, HIV-induced apoptotic cell death has been demonstrated not only *in vitro* but also, more importantly, in infected individuals (Ameisen, J.C., *AIDS 8*:1197-1213 (1994) ; Finkel, T.H., and Banda, N.K., *Curr. Opin. Immunol. 6*:605-615(1995); Muro-Cacho *et al., J. Immunol. 154*:5555-5566 (1995)). Furthermore, apoptosis and $CD4^+$ T-lymphocyte depletion is tightly correlated in different animal models of AIDS (Brunner *et al., Nature 373*:441-444 (1995); Gougeon *et al., AIDS Res. Hum. Retroviruses 9*:553-563 (1993)) and, apoptosis is not observed in those animal models in which viral replication does not result in AIDS (Gougeon *et al., AIDS Res. Hum. Retroviruses 9*:553-563 (1993)). Further data indicates that uninfected but primed or activated T lymphocytes from HIV-infected individuals undergo apoptosis after encountering the TNF-family ligand FasL. Using monocytic cell lines that result in death following HIV infection, it has been demonstrated that infection of U937 cells with HIV results in the *de novo* expression of FasL and that FasL mediates HIV-induced apoptosis (Badley *et*

al., *J. Virol.* *70*:199-206 (1996)). Further the TNF-family ligand was detectable in uninfected macrophages and its expression was upregulated following HIV infection resulting in selective killing of uninfected CD4+ T-lymphocytes (Badley *et al.*, *J. Virol.* *70*:199-206 (1996)).

In rejection of an allograft, the immune system of the recipient animal has not previously been primed to respond because the immune system for the most part is only primed by environmental antigens. Tissues from other members of the same species have not been presented in the same way that, for example, viruses and bacteria have been presented. In the case of allograft rejection, immunosuppressive regimens are designed to prevent the immune system from reaching the effector stage. However, the immune profile of xenograft rejection may resemble disease recurrence more than allograft rejection. In the case of disease recurrence, the immune system has already been activated, as evidenced by destruction of the native islet cells. Therefore, in disease recurrence the immune system is already at the effector stage. Antagonists of the present invention are able to suppress the immune response to both allografts and xenografts by decreasing the rate of TR8 mediated lymphocyte proliferation and differentiation. Such antagonists include the TR8-Fc fusion protein described in Example 5. Thus, the present invention further provides a method for suppression of immune responses.

In addition, TNF-α has been shown to prevent diabetes in strains of animals which are prone to this affliction resulting from autoimmunity. *See* Porter, A., *Tibtech* *9*:158-162 (1991). Thus, agonists and antagonists of the present invention may be useful in the treatment of autoimmune diseases such as type 1 diabetes.

In addition, the role played by the TR8 receptors in cell proliferation, survival and differentiation indicates that agonist or antagonist of the present invention may be used to treat disease states involving aberrant cellular expression of these receptors. TR8 receptors may in some circumstances induce an inflammatory response, and antagonists may be useful reagents for blocking this response. Thus TR8 receptor antagonists (e.g., soluble forms of the TR8 receptors; neutralizing antibodies) may be

useful for treating inflammatory diseases, such as rheumatoid arthritis, osteoarthritis, psoriasis, septicemia, and inflammatory bowel disease.

Antagonists to the TR8 receptor may also be employed to treat and/or prevent septic shock, which remains a critical clinical condition. Septic shock results from an exaggerated host response, mediated by protein factors such as TNF and IL-1, rather than from a pathogen directly. For example, lipopolysaccharides have been shown to elicit the release of TNF leading to a strong and transient increase of its serum concentration. TNF causes shock and tissue injury when administered in excessive amounts. Accordingly, it is believed that antagonists to the TR8 receptor will block the actions of TNF and treat/prevent septic shock. These antagonists may also be employed to treat meningococcemia in children which correlates with high serum levels of TNF.

Among other disorders which may be treated by the antagonists to TR8 receptors, there are included, inflammation which is mediated by TNF receptor ligands, and the bacterial infections cachexia and cerebral malaria. The TR8 receptor antagonists may also be employed to treat inflammation mediated by ligands to the receptor such as TNF.

Modes of administration

The agonist or antagonists described herein can be administered *in vitro, ex vivo*, or *in vivo* to cells which express the receptor of the present invention. By administration of an "effective amount" of an agonist or antagonist is intended an amount of the compound that is sufficient to enhance or inhibit a cellular response to a TNF-family ligand and include polypeptides. In particular, by administration of an "effective amount" of an agonist or antagonists is intended an amount effective to enhance or inhibit TR8 receptor mediated activity. Of course, where cell proliferation and/or differentiation is to be enhanced, an agonist according to the present invention can be co-administered with a TNF-family ligand. One of ordinary skill will appreciate that effective amounts of an agonist or antagonist can be determined

empirically and may be employed in pure form or in pharmaceutically acceptable salt, ester or pro-drug form. The agonist or antagonist may be administered in compositions in combination with one or more pharmaceutically acceptable excipients (i.e., carriers).

It will be understood that, when administered to a human patient, the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon factors well known in the medical arts.

As a general proposition, the total pharmaceutically effective amount of a TR8 polypeptide administered parenterally per dose will be in the range of about 1 μg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the TR8 polypeptide is typically administered at a dose rate of about 1 μg/kg/hour to about 50 μg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

Pharmaceutical compositions are provided comprising an agonist (including TR8 receptor polynucleotides or polypeptides of the invention) or agonist (e.g., TR8 polypeptides of the invention or antibodies thereto) of TR8 and a pharmaceutically acceptable carrier or excipient, which may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. In one embodiment "pharmaceutically acceptable carrier" means a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. In a specific embodiment, "pharmaceutically acceptable" means approved by a regulatory agency of the federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more

particularly humans. Nonlimiting examples of suitable pharmaceutical carriers according to this embodiment are provided in “Remington’s Pharmaceutical Sciences” by E.W. Martin, and include sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glyceral solutions can be employed as liquid carriers, particularly for injectable solutions.

The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Example 1: Expression and Purification of TR8 in E. coli

The bacterial expression vector pQE60 is used for bacterial expression in this example. (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311). pQE60 encodes ampicillin antibiotic resistance ("Amp^r") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra,* and suitable single restriction enzyme cleavage sites. These elements are arranged such that a DNA fragment encoding a polypeptide may be inserted in such as way as to produce that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide. However, in this example, the polypeptide coding sequence is inserted such that translation of the six His codons is prevented and, therefore, the polypeptide is produced with no 6 X His tag.

The DNA sequence encoding the desired portion of the TR8 protein lacking the hydrophobic leader sequence is amplified from the deposited cDNA clone using PCR oligonucleotide primers which anneal to the amino terminal sequences of the desired portion of the TR8 protein and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector are added to the 5' and 3' sequences, respectively.

For cloning the soluble extracellular domain of the TR8 protein, the 5' primer has the sequence:

5' CGCCCATGGCTTTGCAGATCGCTCCTC 3' (SEQ ID NO:7) containing the underlined NcoI restriction site followed by 18 nucleotides complementary to the amino terminal coding sequence of the extracellular domain of theTR8 sequence in FIGS. 1A-C (nucleotides 124-142 of SEQ ID NO:1). One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a desired portion of the complete protein shorter or longer than the mature form. The 3' primer for the soluble extracellular domain has the sequence:

5' CGCAAGCTTTTAGGGCAAGTAAACATG 3' (SEQ ID NO:8) containing the underlined HindIII restriction site followed by 18 nucleotides complementary to the 3' end of the nucleotide sequence shown in FIGS. 1A-C (nucleotides 667-681 in SEQ ID NO:1) encoding the extracellular domain of the TR8 receptor.

The amplified TR8 DNA fragments and the vector pQE60 are digested with NcoI and HindIII and the digested DNAs are then ligated together. Insertion of the TR8 DNA into the restricted pQE60 vector places the TR8 protein coding region including its associated stop codon downstream from the IPTG-inducible promoter and in-frame with an initiating AUG. The associated stop codon prevents translation of the six histidine codons downstream of the insertion point.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook *et al., Molecular Cloning: a Laboratory Manual, 2nd Ed.;* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). *E. coli* strain Ml5/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kan^r"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing TR8 protein, is available commercially from QIAGEN, Inc., *supra.* Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 μg/ml) and kanamycin (25 μg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-b-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

The cells are then stirred for 3-4 hours at 4°C in 6 M guanidine-HCl, pH 8. The cell debris is removed by centrifugation, and the supernatant containing the TR8 is dialyzed against 50 mM Na-acetate buffer pH 6, supplemented with 200 mM NaCl. Alternatively, the protein can be successfully refolded by dialyzing it against 500 mM NaCl, 20% glycerol, 25 mM Tris/HCl pH 7.4, containing protease inhibitors. After renaturation the protein can be purified by ion exchange, hydrophobic interaction and size exclusion chromatography. Alternatively, an affinity chromatography step such as an antibody column can be used to obtain pure TR8 protein. The purified protein is stored at 4°C or frozen at -80°C.

Example 2

Example 2(a): Cloning and Expression of a Soluble Fragment of TR8 Protein in a Baculovirus Expression System

In this example, the plasmid shuttle vector pA2 GP was used to insert the cloned DNA encoding the mature extracellular domain of the TR8 receptor protein shown in FIGS. 1A-C, lacking its naturally associated secretory signal (leader) sequence, into a baculovirus. This protein was expressed using a baculovirus leader and standard methods as described in Summers *et al.*, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by the secretory signal peptide (leader) of the baculovirus gp67 protein and convenient restriction sites such as BamHI, XbaI and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that expresses the cloned polynucleotide.

Many other baculovirus vectors could be used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow *et al.*, *Virology 170*:31-39.

The cDNA sequence encoding essentially the extracellular domain with leader (amino acids 1 to 211 shown in FIGS. 1A-C) of the TR8 receptor protein in the

deposited clone (ATCC Deposit Number 97956) is amplified using PCR oligonucleotide primers corresponding to the relevant 5' and 3' sequences of the gene. The 5' primer for the above has the sequence:

5' CGCGGATCCGCCATCATGGCCCCGCGCGCCCGGC 3' (SEQ ID NO:9) containing the underlined BamHI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol.* *196*:947-950 (1987), followed by 15 bases of the coding sequence of the TR8 protein shown in FIGS. 1A-C (nucleotides 49-67 in SEQ ID NO:1). The 3' primer has the sequence:

5' CGCGGTACCTTAGGGCAAGTAAACATG 3' (SEQ ID NO:10) containing the underlined Asp718 restriction sites followed by 17 nucleotides complementary to the coding sequence in FIGS. 1A-C (nucleotides 667-681 in SEQ ID NO:1).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment was then digested with BamHI and Asp718 and purified on a 1% agarose gel. This fragment is designated herein "F1".

The plasmid is digested with the restriction enzymes BamHI and Asp718 dephosphorylated using calf intestinal phosphatase. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is designated herein "V1".

Fragment F1 and the dephosphorylated plasmid V1 are ligated together with T4 DNA ligase. *E. coli* HB101 cells are transformed with the ligation mixture and spread on culture plates. Other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) may also be used. Bacteria are identified that contain the plasmid with the human TR8 sequences using the PCR method, in which one of the above primers is used to amplify the gene and the second primer is from well within the vector so that only those bacterial colonies containing TR8 gene fragments

show amplification of the DNA. The sequence of the cloned fragment is confirmed by DNA sequencing. The plasmid is designated herein pBacTR8-T.

Five μg of pBacTR8-T is co-transfected with 1.0 μg of a commercially available linearized baculovirus DNA ("BaculoGold baculovirus DNA", Pharmingen, San Diego, CA.), using the lipofection method described by Felgner *et al., Proc. Natl. Acad. Sci. USA 84*:7413-7417 (1987). 1 μg of BaculoGold virus DNA and 5 μg of plasmid pBacTR8-T are mixed in a sterile well of a microtiter plate containing 50 μl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 μl Lipofectin plus 90 μl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution is removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10). After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 μl of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. The recombinant virus is called V-TR8-T.

To verify the expression of the gene used, Sf9 cells are grown in Grace's medium supplemented with 10% heat inactivated FBS. The cells are infected with the recombinant baculovirus V-TR8-T at a multiplicity of infection ("MOI") of about 2. Six hours later the medium is removed and replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). Forty-two hours later, 5 μCi of ^{35}S-methionine and 5 μCi ^{35}S-cysteine (available from Amersham) are added to radiolabel proteins. The cells are further incubated for 16 hours and then they are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography. Microsequencing of the amino acid sequence of the amino terminus of purified protein is used to determine the amino terminal sequence of the mature protein and thus the cleavage point and length of the secretory signal peptide.

Example 2(b): Cloning and Expression of the Full-Length Gene for TR8 Protein in a Baculovirus Expression System

Similarly to the cloning and expression of the truncated version of the TR8 receptor described in Example 2(a), recombinant baculoviruses were generated which express the full length TR8 receptor protein shown in FIGS. 1A-C (SEQ ID NO:2).

In this example, the plasmid shuttle vector pA2 is used to insert the cloned DNA encoding the complete protein, including its naturally associated secretary signal (leader) sequence, into a baculovirus to express the mature TR8 protein. Other attributes of the pA2 vector are as described for the pA2 GP vector used in Example 2(a).

The cDNA sequence encoding the full length TR8 protein in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence shown in FIGS. 1A-C (SEQ ID NO:2), is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer could have the same sequence used in Example 2(a), above. A suitable 3' primer for this purpose has the sequence:

5' CGCGGTACCCTGCGAGTTTGAGGAGTG 3' (SEQ ID NO:11) containing the underlined Asp718 restriction sites followed by 17 nucleotides complementary to the coding sequence in FIGS. 1A-C (nucleotides 2138-2155 in SEQ ID NO:1).

The amplified fragment is isolated and digested with restriction enzymes as described in Example 2(a) to produce plasmid pBacTR8

5 μg of pBacTR8 is co-transfected with 1 μg of BaculoGold (Pharmingen) viral DNA and 10 μl of Lipofectin (Life Technologies, Inc.) in a total volume of 200 μl serum free media. The primary viruses are harvested at 4-5 days post-infection (pi), and used in plaque assays. Plaque purified viruses are subsequently amplified and frozen, as described in Example 2(a).

For radiolabeling of expressed proteins, Sf9 cells are seeded in 12 well dishes with 2.0 ml of a cell suspension containing 0.5 x 10^6 cells/ml and allowed to attach for 4 hours. Recombinant baculoviruses are used to infect the cells at an MOI of 1-2. After 4 hours, the media is replaced with 1.0 ml of serum free media depleted for methionine and cysteine (-Met/-Cys). At 3 days pi, the culture media is replaced with 0.5 ml -Met/-Cys containing 2 μCi each [^{35}S]-Met and [^{35}S]-Cys. Cells are labeled for 16 hours after which the culture media is removed and clarified by centrifugation (Supernatant). The cells are lysed in the dish by addition of 0.2 ml lysis buffer (20 mM HEPES, pH 7.9; 130 mM NaCl; 0.2 mM EDTA; 0.5 mM DTT and 0.5% vol/vol NP-40) and then diluted up to 1.0 ml with dH_2O (Cell Extract). 30 μl of each supernatant and cell extract are resolved by 15% SDS-PAGE. Protein gels are stained, destained, amplified, dried and autoradiographed. Labeled bands corresponding to the recombinant proteins are visible after 16-72 hours exposure.

Example 3: Cloning and Expression of TR8 in Mammalian Cells

A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the

transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRS) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include, human HeLa 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy *et al., Biochem J. 227*:277-279 (1991); Bebbington *et al., Bio/Technology 10:*169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen *et al., Molecular and Cellular Biology*, 438447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart *et al., Cell 41*:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors

contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Example 3(a): Cloning and Expression in COS Cells

The expression plasmid, pTR8 HA, is made by cloning a cDNA encoding the soluble extracellular portion of the TR8 protein into the expression vector pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.).

The expression vector pcDNAI/amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron; (5) several codons encoding a hemagglutinin fragment (i.e., an "HA" tag to facilitate purification) followed by a termination codon and polyadenylation signal arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson *et al., Cell 37:*767 (1984). The fusion of the HA tag to the target protein allows easy detection and recovery of the recombinant protein with an antibody that recognizes the HA epitope. pcDNAIII contains, in addition, the selectable neomycin marker.

A DNA fragment encoding a TR8 protein is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The plasmid construction strategy is as follows. The TR8 cDNA of the deposited clone is amplified using primers that contain convenient restriction sites, much as described above for construction of vectors for expression of TR8 in *E. coli.* Suitable primers include the following, which are used in this example. The 5' primer, containing the underlined BamHI site, a Kozak sequence, an AUG start codon and 6

additional codons of the 5' coding region of the complete TR8 has the following sequence:

5' CGCGGATCCGCCATCATGGCCCCGCGCGCCCGGC 3' (SEQ ID NO:9). The 3' primer has the sequence:

5' CGCTCTAGATCAAGCGTAGTCTGGGACGTCGTATGGGTATTAG GGCAAGTAAACATG 3' (SEQ ID NO:12) containing the underlined XbaI restriction site followed by a stop codon, a sequence encoding a 6x his tag, and 15 nucleotides complementary to the coding sequence in FIGS. 1A-C (nucleotides 667-681 in SEQ ID NO:1).

The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with BamHI and XbaI and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis or other means for the presence of the TR8-encoding fragment.

For expression of recombinant TR8, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook *et al., Molecular Cloning: a Laboratory Manual,* Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under conditions for expression of TR8 by the vector.

Expression of the TR8-HA fusion protein is detected by radiolabeling and immunoprecipitation, using methods described in, for example Harlow *et al., Antibodies: A Laboratory Manual, 2nd Ed.;* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing ^{35}S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson *et al.* cited above. Proteins are precipitated

from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

Example 3(b): Cloning and Expression in CHO Cells

The vector pC4 is used for the expression of TR8 protein. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F. W., Kellems, R. M., Bertino, J. R., and Schimke, R. T., 1978, *J Biol. Chem. 253*:1357-1370, Hamlin, J. L. and Ma, C. 1990, *Biochem. et Biophys. Acta, 1097*:107-143, Page, M. J. and Sydenham, M.A. 1991, *Biotechnology 9*:64-68). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach may be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, *et al., Molecular and Cellular Biology,* March 1985:438-447) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart *et al., Cell 41*:521-530 (1985)). Downstream of the promoter are BamHI, XbaI, and

Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human -actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the TR8 protein in a regulated way in mammalian cells (Gossen, M., & Bujard, H. 1992, *Proc. Natl. Acad. Sci. USA 89*: 5547-5551). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with the restriction enzymes BamHI and Asp718 and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the complete TR8 protein including its leader sequence is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene having, for instance, the same sequences as the 5' and 3' primers used for cloning in baculovirus pA vectors as shown in Example 2, above.

The amplified fragment is digested with the endonucleases BamHI and Asp718 and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene are used for transfection. 5 µg of the expression plasmid pC4 is cotransfected with 0.5 µg of the plasmid pSV2-neo using lipofectin (Felgner *et al., supra)*. The plasmid pSV2neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme

that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of metothrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 µM, 2 µM, 5 µM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 µM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

Example 4

Tissue distribution of TR8 mRNA expression

Northern blot analysis is carried out to examine TR8 gene expression in human tissues, using methods described by, among others, Sambrook *et al.*, cited above. A cDNA probe containing the entire nucleotide sequence of the TR8 protein (SEQ ID NO:1) is labeled with ^{32}P using the *redi*prime DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using a CHROMA SPIN- 100 column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for TR8 mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and are examined with the labeled probe using ExpressHyb hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and films developed according to standard procedures.

Example 5

Example 5(a): Expression and Purification of TR8-Fc(TR8-Ig Fusion Protein) and Cleaved TR8

The putative transmembrane domain of translated TR8 receptor was determined by hydrophobicity using the method of Goldman *et al.* (*Ann. Rev. of Biophys. Biophys. Chem. 15*:321-353 (1986)) for identifying nonpolar transbilayer helices. The region upstream of this transmembrane domain, encoding the putative leader peptide and extracellular domain, is selected for the production of an Fc fusion protein. Primers are designed to PCR the corresponding coding region from the deposited clone with the addition of a BglII site, a Factor Xa protease site and an Asp718I site at the 3 end. PCR with this primer pair results in one band of the expected size. This is cloned into COSFclink to give the TR8-Fclink plasmID The PCR product is digested with EcoRI and Asp718I and ligated into the COSFclink plasmid (Johansen, *et al., J. Biol. Chem. 270*:9459-9471 (1995)) to produce TR8-Fclink.

COS cells are transiently transfected with TR8-Fclink and the resulting supernatant is immunoprecipitated with protein A agarose. Western blot analysis of the immunoprecipitate using goat anti-human Fc antibodies reveals a strong band consistent with the expected size for glycosylated TR8-Fc (greater than 65,940 kD). A 15L transient COS transfection is performed and the resulting supernatant is purified. The purified protein is used to immunize mice following DNA injection for the production of mAbs.

CHO cells are transfected with TR8-Fclink to produce stable cell lines. Five lines are chosen by dot blot analysis for expansion and are adapted to shaker flasks. The line with the highest level of TR8-Fc protein expression is identified by Western blot analysis. TR8-Fc protein purified from the supernatant of this line is used for cell binding studies by flow cytometry, either as intact protein or after factor Xa cleavage and biotinylation.

Example 5(b): Purification of TR8-Fc from CHO E1A Conditioned Media Followed by Cleavage and Biotinylation of TR8.

Assays -- Product purity through the purification is monitored on 15% Laemmli SDS-PAGE gels run under reducing and non-reducing conditions. Protein concentration was monitored by A_{280} assuming extinction coefficients for the receptor and the chimera calculated from the sequences.

Protein G Chromatography of the TR8-Fc Fusion Protein -- All steps described below are carried out at 4°C. 15L of CHO conditioned media (CM) (0.2 μ filtered following harvest in cell culture) is applied to a 5 X 10 cm column of Protein G at a linear flow rate of 199 cm/h. The column is previously washed with 100 mM glycine, pH 2.5 and equilibrated in 20 mM sodium phosphate, 150 mM sodium chloride, pH 7 prior to sample application. After the CM is loaded the column is washed with 5 column volumes of 20 mM sodium phosphate, 150 mM sodium chloride, pH 7 and eluted with 100 mM glycine, pH 2.5. The eluate is immediately neutralized with 3 M Tris, pH 8.5 and 0.2 μ filtered.

Concentration/Dialysis -- Protein G eluate is concentrated about 10 fold in an Amicon stirred cell fitted with a 30K membrane. The concentrate is dialyzed against buffer.

Factor Xa Cleavage and Purification to Generate Free Receptor -- TR8-Fc is added to 50 μg of Factor Xa resulting in a 1:200 e:s ratio. The mixture is incubated overnight at 4°C.

Protein G Chromatography of the Free TR8 receptor--A 1 ml column of Protein G is equilibrated in 20 mM sodium phosphate, 150 mM sodium chloride, pH 6.5 in a disposable column using gravity flow. The cleaved receptor is passed over the

column 3 times after which the column is washed with 20 mM sodium phosphate, 150 mM sodium chloride, pH 6.5 until no A_{280} absorbence is seen. The column is eluted with 2.5 ml of 100 mM glycine, pH 2.5 neutralized with 83 µl of 3 M Tris, pH 8.5. TR8 elutes in the nonbound fraction.

Concentration -- The nonbound fraction from the Protein G column is concentrated in a Centricon 10K cell (Amicon) to about a final concentration of 3.5 mg/ml estimated by A_{280} extinction coefficient 0.7.

Mono S Chromatography -- The concentrated sample is diluted to 5 ml with 20 mM sodium phosphate, pH 6 and applied to a 0.5 X 5 cm Mono S column equilibrated in 20 mM sodium phosphate, pH 6 at a linear flow rate of 300 cm/h. The column is washed with 20 mM sodium phosphate, pH 6 and eluted with a 20 column volume linear gradient of 20 mM sodium phosphate, pH 6 to 20 mM sodium phosphate, 1 M sodium chloride, pH 6. TR8 protein elutes in the nonbound fraction.

Concentration/Dialysis -- The nonbound fraction from the Mono S column is concentrated to 1 ml as above using a Centricon 10K cell and is dialyzed against 20 mM sodium phosphate, 150 mM sodium chloride, pH 7.

Biotinylation -- 0.5 mg of TR8 at about 1-2 mg/ml is dialyzed against 100 mM borate, pH 8.5. A 20-fold molar excess of NHS-LC Biotin is added and the mixture is left on a rotator overnight at 4°C. The biotinylated TR8 is dialyzed against 20 mM sodium phosphate, 150 mM sodium chloride, pH 7, sterile filtered and stored at -70°C. Biotinylation is demonstrated on a Western blot probed with strepavidin HRP and subsequently developed with ECL reagent.

***Example 6:** Characterization of the Intracellular Domain of TR8:Interaction with TRAFs and Activation of NF-$_{K}$ B and JNK.*

Various members of the TNF receptor superfamily interact directly with signaling molecules of the TNF receptor-associated factor (TRAF) family to elicit activation of NF-κB (nuclear factor κB) and the c-jun N-terminal kinase (JNK/SAPK) pathway. TR8, a TNF receptor family member and its ligand (TR8L) promotes survival of dendritic cells and differentiation of osteoclasts. TR8 contains 383 amino acids in its intracellular domain (amino acid residues 234-615; amino acid residues 209-590 of SEQ ID NO:2) in which resides three putative TRAF binding domains (termed I, II, and III). In this study, we examined the region of TR8 needed for interaction with TRAF molecules and for stimulation of NF-κB and JNK activity. We constructed epitope-tagged TR8 (F- TR8-615) and three C-terminal truncations (F-TR8-330, 427, and 530) lacking 85, 188, and 285 amino acids respectively. From this deletion analysis, TRAF2, TRAF5, and TRAF6 interact with TR8 at its C-terminal 85 amino acid tail, although the binding affinity appears to be in the order of TRAF2 >>> TRAF5 >TRAF6. Furthermore, overexpression of TR8 stimulates JNK and NF-κB activation. However, when the C-terminal tail which is necessary for TRAF binding was deleted, the truncated TR8 receptor was still capable of stimulating JNK activity, but not NF-κB, suggesting that interaction with TRAFs is necessary for NF-κB, but not necessarily for activation of the JNK pathway.

To date, over 20 members of the TNF ligand and receptor superfamilies have been identified. Most of these receptors activate signaling cascades including activation of NF-κB, protein kinases (MAPK/JNK/p38), and apoptosis through engagement of various adaptor proteins (Liu et al., *Cell*, 87:565-576 (1996); Darnay et al., *J. Leuk. Biol.*, 61:559-566 (1977); Song et al., *Proc. Natl. Acad. Sci.* 94:9792-9796 (1997)). Activation of apoptosis is typically transmitted through death domain containing receptors. Additionally, many of the TNFR family members activate NF-κB and JNK pathways via interaction with various TRAF family members (Liu et al., *Cell* 87:565-576 (1996); Song et al., *Proc. Natl. Acad. Sci.* 94:9792-9796 (1997); Cao et al., *Nature* 383:443-446 (1996); Hsu et al., *J. Biol. Chem.* 272:13471-13474 (1996);

Ishida et al., *J. Biol. Chem.* 271:28745-28748 (1996); Marsters et al., *J. Biol. Chem.* 272:14029-14032 (1997); Rothe et al., *Cell* 78:681-692 (1994); Reinhard et al., *EMBO J.* 16:1080-1092 (1997); Natoli et al., *Science* 275:200-203 (1997); Rothe et al., *Science* 269:1424-1427 (1995)). The TRAF family consists of six distinct proteins which contain a Ring and zinc finger motif in their N-terminus and a C-terminal domain which appears to be responsible for self-association and protein interaction. TRAF family members TRAF1, TRAF2, and TRAF3 bind to distinct motifs within CD40, CD30, ATAR/HVEM, and p80 TNFR (Hsu et al., *J. Biol. Chem.* 272:13471-13474 (1996); Ishida et al., *J. Biol. Chem.* 271:28745-28748 (1996); Marsters et al., *J. Biol. Chem.* 272:14029-14032 (1997); Boucher et al., *Biochem. Biophys. Res. Comm.* 233:592-600 (1997)). The PXQXT/S motif is characteristic for binding TRAF1, TRAF2, and TRAF5 (Hsu et al., *J. Biol. Chem.* 272:13471-13474 (1996); Ishida et al., *J. Biol. Chem.* 271:28745-28748 (1996)). Moreover, TRAF6 interacts with CD40 via a 15 amino acid region (residues 230-245) (Ishida et al., *J. Biol. Chem.* 271:28745-28748 (1996)). Of these TRAF molecules, only TRAF2, TRAF5, and TRAF6 have been demonstrated to mediate signaling of NF-κB and JNK (Song et al., *Proc. Natl. Acad. Sci.* 94:9792-9796 (1997); Cao et al., *Nature* 383:443-446 (1996); Reinhard et al., *EMBO J.* 16:1080-1092 (1997); Natoli et al., *Science* 275:200-203 (1997)).

To further elucidate regions of the intracellular domain necessary for signaling by TR8, we constructed various C-terminal truncations of TR8 and transiently expressed them in human cultured cell lines to characterize their ability to activate JNK and NF-κB and for their ability to interact with various TRAF family members. From this deletion analysis, TRAF2, TRAF5, and TRAF6 interact with TR8 at its C-terminal 85 amino acid tail, although TRAF2 appears to bind preferentially. Furthermore, overexpression of TR8 stimulates JNK and NF-κB activation. However, when the C-terminal tail, which is necessary for TRAF binding, was deleted, the truncated TR8 receptor was still capable of stimulating JNK activity, but not NF-κB. These results

suggest that TR8's interaction with TRAFs is necessary for NF-κB, but not for activation of the JNK pathway.

Experimental Procedures

Reagents, Cell lines, and Antibodies -- HeLa, an epithelial carcinoma cell line, and 293, a human embryonic kidney cell line, were obtained from the American Type Culture Collection (Rockville, MD) and cultured in MEM supplemented with 10% fetal bovine serum and antibiotics. Affinity-purified rabbit anti-TRAF2 (SC-876, C-20) and anti-JNK1 (SC-474, C-17) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Goat anti-rabbit IgG-conjugated horseradish peroxidase was obtained from BioRad Laboratories (Hercules, CA). Anti-FLAG (monoclonal antibody M2) and anti-FLAG (M2) conjugated-agarose were obtained Eastman Kodak Co. (New Haven, CT). Goat anti-mouse IgG conjugated to horseradish peroxidase was obtained from Transduction Laboratories (Lexington, KY). Protein A/G sepharose was obtained from Pierce (Rockford, IL).

Expression Plasmids -- The complete cDNA for TR8 (pSPORT3.0-TR8) was identified through a homology search of an expressed sequence tag (EST) cDNA database (Human Genome Sciences, Inc., Rockville, MD) obtained from a primary dendritic cell cDNA library for proteins containing the cysteine-rich repeat characteristic of TNFR family members. To generate FLAG-tagged TR8-615, primers (5'-primer: CTAAGAAAGCTTTGTACCAGTGAGAAGCAT (SEQ ID NO:13) and 3'-primer: GACGTAGTCGACTCAAGCCTTGGCCCCGCC (SEQ ID NO:14) were used in a PCR reaction with pSPORT3.0-TR8 to generate a PCR product that would encode residues 33-615 (lacking the signal sequence) and cloned into the HindIII/SalI site of the expression vector pCMVFLAG1 (Eastman Kodak Co., New Haven, CT). TR8 deletion mutants were generated by PCR using the above 5' primer and the 3' primers (TR8-330:TCCTACGTCGACTCAGCTGACCAATGAG AGAGCATCCT (SEQ ID NO:15); TR8-427: AACGGCGTCGACTCAACTGTC

CACCTCTTTTTGCAA (SEQ ID NO:16); and TR8-530: CGCTGAGTCGACT CAGGAGTTACTTGTTTCCAGTCAC (SEQ ID NO:17)) and cloned into the HindIII/SalI site of pCMVFLAG1. All plasmids were verified by automated DNA sequencing. The complete cDNA for TRAF2 was cloned by PCR using primers containing BamHI (5') and SalI (3') sites and pcDNA3HisTRAF2 as a template. The TRAF2 PCR product was digested with BamHI/SalI and cloned into pRKmyc resulting in pRKmycTRAF2. The cDNA for TRAF6 was digested from pSRα-TRAF6 with KpnI/EcoRI and cloned into pBS(KS-) to give rise to pBS-TRAF6.

In Vitro Translation of ^{35}S-Labeled TRAFs -- Expression vectors encoding for TRAF2 (pRKmycTRAF2), TRAF5 (pcDNA3mycTRAF5), and TRAF6 (pBS-TRAF6) were *in vitro* transcribed and translated with ^{35}S-Met (Amersham, Chicago, IL) using the TNT system as described by the manufacturer (Promega, Madison, WI).

Transient Transfections -- HeLa (1.5 x 10^6 cells/100 mm dish) and 293 (2 x 10^6 cells/100 mm dish) cells were plated the day before and transfected with 7.5-10 μg of expression vector by using Lipofectamine (GIBCO BRL, Gaithersburg, MD) as described by the manufacturer and allowed to proceed for an additional 24 hrs. Alternatively, 293 cells (0.6 x 10^6 cells/well, 6-well plate) were plated the day before and transfected the next day by calcium phosphate as described by the manufacturer (GIBCO BRL, Gaithersburg, MD). Cells were harvested 36-40 hrs post-transfection and half of the cells were analyzed for expression of epitope-tagged receptors and JNK activities and the other half of the cells were analyzed for NF-κB by EMSAs. Lysates were prepared in lysis buffer (20 mM TRIS pH 8, 250 mM NaCl, 1 mM DTT, 2 mM EDTA, 1% Triton X-100, 10 g/ml leupeptin, 10 g/ml aprotinin, 0.5 mg/ml benzamidine, and 2 mM sodium vanadate). After 30 min. on ice, the samples were cleared by centrifugation for 10 min. Protein was estimated using a BioRad Protein determination kit (BioRad, Hercules, CA).

Western Blotting -- Whole cell lysates (15g) or proteins from immunoprecipitations were separated by 8.5% SDS-PAGE and electroblotted onto

nitrocellulose membranes (BioRad, Hercules, CA). Western blot analysis was performed using the indicated antibodies, and membranes were developed by Enhanced Chemiluminescence (ECL) (Amersham, Chicago, IL).

Immunoprecipitations and JNK Kinase Assays -- From transient transfected cells, lysates were prepared and immunoprecipitated using anti-FLAG-conjugated agarose or anti-JNK1 and protein A/G sepharose for 1 hr. Where indicated, ^{35}S-labeled proteins were added to the lysate prior to immunoprecipitation. Beads were collected by centrifugation and washed four times in lysis buffer followed by two washes in kinase buffer (20 mM TRIS, pH 8, 50 mM NaCl, and 1 mM DTT). For coimmunoprecipation, proteins were eluted in SDS-sample buffer, boiled, and subjected to SDS-PAGE. Analysis of JNK activity was performed using exogenously added GST-Jun(1-79) as a substrate as previously described (Haridas et al., *J. Immunol.* 160:3152-3162 (1998)). Quantitation of JNK activity and ^{35}S-labeled TRAF binding was analyzed using a PhosphoImager and Imagequant Software (Molecular Dynamics, Sunnyvale, CA).

Electrophoretic Mobility Shift Assays (EMSA) — Nuclear extracts were prepared from transfected cells essentially as described (Haridas et al., *J. Immunol.* 160:3152-3162 (1998)). Equivalent amounts of nuclear protein were used in a EMSA reaction with ^{32}P-labeled NF-κB oligonucleotide from the HIV-LTR as described (Haridas et al., *J. Immunol.*, 160:3152-3162 (1998)). Quantitation of relative NF-κB activation was analyzed using a PhosphoImager and Imagequant Software.

Results And Discussion

The full length TR8 encoding cDNA encodes a protein of 615 amino acid residues. The extracellular domain (residues 1-208 of FIGS. 1A-C; residues -25 to 183 of SEQ ID NO:2)) contains a signal sequence and the conserved cysteine rich repeats characteristic of the TNFR family (Vandenabeele et al., *Trends Cell Biol.* 5:392-399 (1995)). The intracellular domain (residues 234-615 of FIGS. 1A-C; residues 209-590

of SEQ ID NO:2) is the largest of all the TNFR family members to date and contains no homology to other members of this family.

Construction and Expression of Epitope-Tagged TR8 -- To facilitate detection and immunoprecipitation of TR8 in cultured cells, we constructed a FLAG epitope-tagged version of TR8 in the plasmid pCMVFLAG1. The mature polypeptide encoding residues 33-615 of FIGS. 1A-C; residues 8-590 of SEQ ID NO:2 (F-TR8-615) would be directed to the plasma membrane with a FLAG epitope tag at its N-terminus (FIG. 5A). To initially identify which region of the cytoplasmic domain is needed for signaling, we constructed three C-terminal deletions designated F-TR8-530, 427, and 330 (FIGS. 1A-C) lacking 85, 188, and 285 amino acids, respectively.

Most of the TNFR family members interact directly with various members of the TRAF family of signaling proteins. Of those receptors that bind to TRAF2, TRAF3, and TRAF5, a consensus TRAF binding motif (PxQxT/S) in the receptor is necessary for TRAF interaction (Ishida et al., *J. Biol. Chem.* 271:28745-28748 (1996); Boucher *et al., Blochem. Biophys. Res. Comm. 233:592-600 (1997);* Ishida et al., *Proc. Natl. Acad. Sci.* 93:9437-9442 (1996); Brodeur et al., *J. Biol. Chem.* 272:19777-19784 (1997)). By inspection of the intracellular domain of TR8, there appears to be three potential TRAF binding domains, two at the C-terminus (TRAFII and III) and one in the middle of the intracellular domain (TRAFIII) (FIG. 5B). Transient expression of F-TR8 and its deletion mutants was demonstrated in both HeLa, an epithelial carcinoma cell line, and 293, a human embryonic kidney cell line (data not shown). As expected, the deletion mutants were expressed similarily in both cell lines tested; however, expression levels of the deletion mutants was typically less than the full length receptor even using similar amounts of expression vectors.

TRAF2, TRAF5, and TRAF6 Interact with the C-Terminus of TR8 -- Since most of the TNFR family members utilize TRAFs as signaling components and that TR8 contains putative TRAF binding domains, we examined the ability for TR8 to interact with various TRAFs. We transiently transfected HeLa and 293 cells with vectors directing expression of F-TR8-615 and F-TR8 deletion mutants. After 24-36 hr, cell

lysates were prepared and epitope-tagged receptors were immunoprecipitated with anti-FLAG conjugated-agarose. Coprecipitation of endogenous TRAF2 was detected by western blotting with anti-TRAF2 polyclonal antibodies. When expressed in HeLa and 293 cells, only F-TR8-615 routinely precipitated endogenous TRAF2, while none of the F-TR8 deletion mutants could precipitate endogenous TRAF2 (data not shown). Membranes were also probed with anti-FLAG to insure precipitation of epitope-tagged receptors (data not shown).

To examine whether other TRAFs could interact with TR8, we transiently transfected 293 cells with F-TR8 expression vectors. After 36 hr, cell lysates were prepared and *in vitro* translated ^{35}S-labeled TRAF2, TRAF5, and TRAF6 were added to each of the lysates. The epitope-tagged receptors were immunoprecipitated with anti-FLAG conjugated-agarose and bound proteins were eluted in SDS-sample buffer and subjected to SDS-PAGE. The bound ^{35}S-labeled TRAFs were detected by exposure of the dried SDS-PAGE gel to x-ray film. Like coprecipitation of endogenous TRAF2, only F-TR8-615 coprecipitated ^{35}S-labeled TRAF2 and to a lesser extent ^{35}S-labeled TRAF5 and TRAF6 (data not shown). Quantitation of ^{35}S-labeled TRAF2, TRAF5, and TRAF6 bound to F-TR8-615 resulted in a 145-, 11-, and 5-fold increase in binding relative to vector transfected cells, respectively. Thus, we have shown that TRAF2, TRAF5, and TRAF6 interact with TR8 at its C-terminal 85 residues.

TR8 Deletion Mutants Lacking TRAF Binding Domains (II and III) Activate JNK -- TRAF2, TRAF5, and TRAF6 are involved in JNK activation (Song et al., *Proc. Natl. Acad. Sci.* 94:9792-9796 (1997)) by various members of the TNFR family and the interleukin-1 receptor (Cao et al., *Nature* 383:443-446 (1996)) (i.e., TRAF6). We tested whether other deletion mutants of TR8 lacking the C-terminus are capable of activation of JNK. Since several TNFR family members are capable of ligand-independent signaling when overexressed in cultured cell lines (Darnay et al., *J. Leuk. Biol.* 61:559-566 (1997)), we transiently transfected 293 cells with increasing amounts of F-TR8 expression vectors. After 36 hr post-transfection, cell lysates were

prepared and analyzed for receptor expression by western blotting with anti-FLAG antibodies (data not shown). Furthermore, the cell lysates were assayed for JNK activation by immune complex kinase assays using GST-Jun(1-79) as a substrate. Transient overexpression of F-TR8-615 in 293 cells leads to activation of JNK. Furthermore, F-TR8-530 and 427 deletion mutants lacking 85 and 188 residues from the C-terminus, respectively, could still activate JNK. However, C-terminal truncation of 285 residues (which leaves approximately 98 amino acids intact) could not activate JNK (data not shown). From at least three independent transfection experiments, we found that F-TR8-615, 530, and 427 could increase JNK activity between 4- to 10-fold, while F-TR8-330 was found not to exceed 1.5-fold relative to vector transfected samples. This data suggests that F-TR8-530 and F-TR8-427 may stimulate JNK activation in the absence of binding directly to TRAFs. Since F-TR8-330 could not stimulate JNK activation, we could tentatively localize a JNK activation domain between residues 330-427 of FIGS. 1A-C (residues 305-402 of SEQ ID NO:2) within the cytoplasmic domain of TR8.

TR8's C-Terminus is Necessary for NF-κB Activation -- Overexpression of TR8 in 293 cells activates NF-κB as analyzed by gel mobility shift assays (Anderson et al., Nature 390:175-179 (1997)). To explore whether TR8 deletions mutants could activate NF-κB, we transiently transfected 293 cells with F-TR8-615 and the F-TR8 deletions mutants. Cells were harvested 36 hr post-transfection and half of the cells were used for JNK and western blotting, while the other half of the cells were used to prepare nuclear extracts. Western blotting with anti-FLAG antibodies indicated expression of the epitope-tagged receptors and JNK immune complex kinase assays indicated stimulation of JNK activity. Analysis of NF-κB by a gel mobility shift assay indicated that only F-TR8-615 could activate NF-κB (data not shown). None of the F-TR8 deletions were capable of activating NF-κB in three independent transient transfection experiments, even though from the same transfections F-TR8-530 and 427 could activate JNK.

Our data is consistent with a previous report (Anderson et al., Nature 390:175-179 (1997), indicating that transient overexpression of TR8 in 293 cells induces NF-κB. We further demonstrated deletion of the C-terminal 85 residues, which is necessary for TRAF interaction, appears also to be necessary for NF-κB activation. Whether the interaction between TR8 and TRAFs is responsible for NF-κB activation remains to be determined. Our data is in agreement with reports which show that TRAF2, TRAF5, and TRAF6 participate in NF-κB activation by other TNFR family members (Song et al., *Proc. Natl. Acad. Sci.* 94:9792-9796 (1997)).

Stimulation of mouse thymocytes or T-cells, but not B-cells, by TR8L/TRANCE induces JNK activation (Wong et al., *J. Biol. Chem.* 272:25190-25194 (1997)) which could be inhibited in thymocytes from transgenic mice expressing a dominant negative form of TRAF2 (Wong et al., *J. Exp. Med.* 186:2075-2080 (1997)). From our deletion analysis of TR8, we provided evidence that TR8 lacking the TRAF binding domain could still stimulate JNK activity. Furthermore, our deletion analysis implicates residues between 330-427 of FIGS. 1A-C (305-402 of SEQ ID NO:2) of TR8 to be necessary for JNK activation. Thus, it appears that TR8 can activate JNK in a TRAF-independent manner. This may be contradictory to that published by ligand stimulation of thymocytes from dominant negative TRAF2 transgenic mice (Wong et al., *J. Exp. Med.* 186:2075-2080 (1997)), however, the experimental conditions are too different to compare these two sets of results. It is possible that TR8 can activate the JNK pathway in both a TRAF-dependent and -independent fashion. Moreover, it is also possible that other unidentified adaptor proteins and TRAF-like molecules are responsible for signaling by TR8.

In summary, TR8 encodes the largest cytoplasmic domain (383 amino acids) of any TNFR family member thus far. For the first time, we provide evidence that TRAF2, TRAF5, and TRAF6 bind to the C-terminal 85 amino acids, however TRAF2 appears to bind better than TRAF5 and TRAF6. Furthermore, we demonstrated that deletion of the TRAF interaction motif at the C-terminus, did not

inhibit TR8 from stimulation of JNK activity, suggesting that TR8 could potentially activate JNK in a TRAF-independent manner. However, deletion of the C-terminal 85 residues results in loss of NF-κB activation. Thus, we have demonstrated that TRAF family members interact with the novel TNFR family member TR8, and could possibly participate in TR8 signal transduction.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: NI, JIAN
MOORE, PAUL

(ii) TITLE OF INVENTION: HUMAN TUMOR NECROSIS FACTOR RECEPTOR-LIKE PROTEIN 8

(iii) NUMBER OF SEQUENCES: 24

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: HUMAN GENOME SCIENCES, INC.
(B) STREET: 9410 KEY WEST AVENUE
(C) CITY: ROCKVILLE
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(E) COUNTRY: US
(F) ZIP: 20850

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(C) REFERENCE/DOCKET NUMBER: PF368

(ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2853 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS

(B) LOCATION: 49.1893

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 49.121

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 124.1893

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GTCGACCCAC GCGTCCGGGC CGCGGCGCCC GCCAGCCTGT CCCGCGCC ATG GCC CCG        57
                                                     Met Ala Pro
                                                     -25

CGC GCC CGG CGC CGC CCG CTG TTC GCG CTG CTG CTG CTC TGC GCG CTG        105
Arg Ala Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu Leu Cys Ala Leu
    -20                 -15                 -10

CTC GCC CGG CTG CAG GTG GCT TTG CAG ATC GCT CCT CCA TGT ACC AGT        153
Leu Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser
 -5                      1               5                  10

GAG AAG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC AAA TGT GAA CCA        201
Glu Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro
                15                  20                  25

GGA AAG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT GAC AGT GTA TGT        249
Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser Asp Ser Val Cys
            30                  35                  40

CTG CCC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG AAT GAA GAA GAT        297
Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp Asn Glu Glu Asp
        45                  50                  55

AAA TGC TTG CTG CAT AAA GTT TGT GAT ACA GGC AAG GCC CTG GTG GCC        345
Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala
 60                  65                  70

GTG GTC GCC GGC AAC AGC ACG ACC CCC CGG CGC TGC GCG TGC ACG GCT        393
Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala
75                  80                  85                  90

GGG TAC CAC TGG AGC CAG GAC TGC GAG TGC TGC CGC CGC AAC ACC GAG        441
Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu
                95                 100                 105

TGC GCG CCG GGC CTG GGC GCC CAG CAC CCG TTG CAG CTC AAC AAG GAC        489
Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp
            110                 115                 120

ACA GTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT GAT GCC TTT TCC        537
Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser
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        125                 130                 135

TCC ACG GAC AAA TGC AGA CCC TGG ACC AAC TGT ACC TTC CTT GGA AAG        585
Ser Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys
    140                 145                 150

AGA GTA GAA CAT CAT GGG ACA GAG AAA TCC GAT GTG GTT TGC AGT TCT        633
Arg Val Glu His His Gly Thr Glu Lys Ser Asp Val Val Cys Ser Ser
155                 160                 165                 170

TCT CTG CCA GCT AGA AAA CCA CCA AAT GAA CCC CAT GTT TAC TTG CCC        681
Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His Val Tyr Leu Pro
                175                 180                 185

GGT TTA ATA ATT CTG CTT CTC TTC GCG TCT GTG GCC CTG GTG GCT GCC        729
Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala Leu Val Ala Ala
            190                 195                 200

ATC ATC TTT GGC GTT TGC TAT AGG AAA AAA GGG AAA GCA CTC ACA GCT        777
Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys Ala Leu Thr Ala
        205                 210                 215

AAT TTG TGG CAC TGG ATC AAT GAG GCT TGT GGC CGC CTA AGT GGA GAT        825
Asn Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg Leu Ser Gly Asp
    220                 225                 230

AAG GAG TCC TCA GGT GAC AGT TGT GTC AGT ACA CAC ACG GCA AAC TTT        873
Lys Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His Thr Ala Asn Phe
235                 240                 245                 250

GGT CAG CAG GGA GCA TGT GAA GGT GTC TTA CTG CTG ACT CTG GAG GAG        921
Gly Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu Thr Leu Glu Glu
                255                 260                 265

AAG ACA TTT CCA GAA GAT ATG TGC TAC CCA GAT CAA GGT GGT GTC TGT        969
Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln Gly Gly Val Cys
            270                 275                 280

CAG GGC ACG TGT GTA GGA GGT GGT CCC TAC GCA CAA GGC GAA GAT GCC       1017
Gln Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln Gly Glu Asp Ala
        285                 290                 295

AGG ATG CTC TCA TTG GTC AGC AAG ACC GAG ATA GAG GAA GAC AGC TTC       1065
Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu Glu Asp Ser Phe
    300                 305                 310

AGA CAG ATG CCC ACA GAA GAT GAA TAC ATG GAC AGG CCC TCC CAG CCC       1113
Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg Pro Ser Gln Pro
315                 320                 325                 330

ACA GAC CAG TTA CTG TTC CTC ACT GAG CCT GGA AGC AAA TCC ACA CCT       1161
Thr Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser Lys Ser Thr Pro
                335                 340                 345

CCT TTC TCT GAA CCC CTG GAG GTG GGG GAG AAT GAC AGT TTA AGC CAG       1209
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Pro Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln
            350                 355                 360

TGC TTC ACG GGG ACA CAG AGC ACA GTG GGT TCA GAA AGC TGC AAC TGC      1257
Cys Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu Ser Cys Asn Cys
        365                 370                 375

ACT GAG CCC CTG TGC AGG ACT GAT TGG ACT CCC ATG TCC TCT GAA AAC      1305
Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met Ser Ser Glu Asn
    380                 385                 390

TAC TTG CAA AAA GAG GTG GAC AGT GGC CAT TGC CCG CAC TGG GCA GCC      1353
Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro His Trp Ala Ala
395                 400                 405                 410

AGC CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC TGC CGG AAC CCT      1401
Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly Cys Arg Asn Pro
                415                 420                 425

CCT GGG GAG GAC TGT GAA CCC CTC GTG GGT TCC CCA AAA CGT GGA CCC      1449
Pro Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro Lys Arg Gly Pro
            430                 435                 440

TTG CCC CAG TGC GCC TAT GGC ATG GGC CTT CCC CCT GAA GAA GAA GCC      1497
Leu Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro Glu Glu Glu Ala
        445                 450                 455

AGC AGG ACG GAG GCC AGA GAC CAG CCC GAG GAT GGG GCT GAT GGG AGG      1545
Ser Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly Ala Asp Gly Arg
    460                 465                 470

CTC CCA AGC TCA GCG AGG GCA GGT GCC GGG TCT GGA ATC TCC CCT GGT      1593
Leu Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly Ile Ser Pro Gly
475                 480                 485                 490

GGC CAG TCC CCT GCA TCT GGA AAT GTG ACT GGA AAC AGT AAC TCC ACG      1641
Gly Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn Ser Asn Ser Thr
                495                 500                 505

TTC ATC TCC AGC GGG CAG GTG ATG AAC TTC AAG GGC GAC ATC ATC GTG      1689
Phe Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly Asp Ile Ile Val
            510                 515                 520

GTC TAC GTC AGC CAG ACC TCG CAG GAG GGC GCG GCG GCG GCT GCG GAG      1737
Val Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala Ala Ala Ala Glu
        525                 530                 535

CCC ATG GGC CGC CCG GTG CAG GAG GAG ACC CTG GCG CGC CGA GAC TCC      1785
Pro Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala Arg Arg Asp Ser
    540                 545                 550

TTC GCG GGG AAC GGC CCG CGC TTC CCG GAC CCG TGC GGC GGC CCC GAG      1833
Phe Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys Gly Gly Pro Glu
555                 560                 565                 570
```

```
GGG CTG CGG GAG CCG GAG AAG GCC TCG AGG CCG GTG CAG GAG CAA GGC       1881
Gly Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val Gln Glu Gln Gly
                575                 580                 585

GGG GCC AAG GCT TGAGCGCCCC CCATGGCTGG GAGCCCGAAG CTCGGAGCCA          1933
Gly Ala Lys Ala
            590

GGGCTCGCGA GGGCAGCACC GCAGCCTCTG CCCCAGCCCC GGCCACCCAG GGATCGATCG    1993

GTACAGTCGA GGAAGACCAC CCGGCATTCT CTGCCCACTT TGCCTTCCAG GAAATGGGCT    2053

TTTCAGGAAG TGAATTGATG AGGACTGTCC CCATGCCCAC GGATGCTCAG CAGCCCGCCG    2113

CACTGGGGCA GATGTCTCCC CTGCCACTCC TCAAACTCGC AGCAGTAATT TGTGGCACTA    2173

TGACAGCTAT TTTTATGACT ATCCTGTTCT GTGGGGGGGG GGGTCTGTTT TCCCCCCATA    2233

TTTGTATTCC TTTTCATAAC TTTTCTTGAT ATCTTTCCTC CCTCTTTTTT AATGTAAAGG    2293

TTTTCTCAAA AATTCTCCTA AAGGTGAGGG TCTCTTTCTT TTCTCTTTTC CTTTTTTTTT    2353

TCTTTTTTTG GCAACCTGGC TCTGGCCCAG GCTAGAGTGC AGTGGTGCGA TTATAGCCCG    2413

GTGCAGCCTC TAACTCCTGG GCTCAAGCAA TCCAAGTGAT CCTCCCACCT CAACCTTCGG    2473

AGTAGCTGGG ATCACAGCTG CAGGCCACGC CCAGCTTCCT CCCCCCGACT CCCCCCCCAG    2533

AGACACGGTC CCACCATGTT AACCCAGCCT GGTCTCAAAC TCACCCAGTA AAGCAGTCCT    2593

ACCAGCCTCG GCCTCCCAAA GTCACTGGGA TTCACAGGCG TGAGCCCCCA CGCTGGCCTG    2653

CTTTACGTAT TTTCTTTTGT GCCCCTGCTC ACAGTGTTTT AGAGATGGCT TTCCCAGTGT    2713

GTGTTCATTG TAAACACTTT TGGGAAAGGG CTAAACATGT GAGGCCTGGA GATAGTTGCT    2773

AAGTTGCTAG GAACATGTGG TGGGACTTTC ATATTCTGAA AAATGTTCTA TATTCTCATT    2833

TTTCTAAAAA AAAAAAAAAA                                                2853
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 615 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ala Pro Arg Ala Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu Leu
-25                 -20                 -15                 -10
```

Cys Ala Leu Leu Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro Pro
-5 1 5

Cys Thr Ser Glu Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn Lys
10 15 20

Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser Asp
25 30 35

Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp Asn
40 45 50 55

Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys Ala
60 65 70

Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys Ala
75 80 85

Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg Arg
90 95 100

Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln Leu
105 110 115

Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser Asp
120 125 130 135

Ala Phe Ser Ser Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr Phe
140 145 150

Leu Gly Lys Arg Val Glu His His Gly Thr Glu Lys Ser Asp Val Val
155 160 165

Cys Ser Ser Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His Val
170 175 180

Tyr Leu Pro Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala Leu
185 190 195

Val Ala Ala Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys Ala
200 205 210 215

Leu Thr Ala Asn Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg Leu
220 225 230

Ser Gly Asp Lys Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His Thr
235 240 245

Ala Asn Phe Gly Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu Thr
250 255 260

Leu Glu Glu Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln Gly
265 270 275

Gly Val Cys Gln Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln Gly

280 285 290 295

Glu Asp Ala Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu Glu
300 305 310

Asp Ser Phe Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg Pro
315 320 325

Ser Gln Pro Thr Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser Lys
330 335 340

Ser Thr Pro Pro Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp Ser
345 350 355

Leu Ser Gln Cys Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu Ser
360 365 370 375

Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met Ser
380 385 390

Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro His
395 400 405

Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly Cys
410 415 420

Arg Asn Pro Pro Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro Lys
425 430 435

Arg Gly Pro Leu Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro Glu
440 445 450 455

Glu Glu Ala Ser Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly Ala
460 465 470

Asp Gly Arg Leu Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly Ile
475 480 485

Ser Pro Gly Gly Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn Ser
490 495 500

Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly Asp
505 510 515

Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala Ala
520 525 530 535

Ala Ala Glu Pro Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala Arg
540 545 550

Arg Asp Ser Phe Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys Gly
555 560 565

Gly Pro Glu Gly Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val Gln
570 575 580

```
Glu Gln Gly Gly Ala Lys Ala
    585                 590
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 450 amino acids
  (B) TYPE: amino acid
  (C) STRANDEDNESS: single
  (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
1               5                   10                  15

Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
            20                  25                  30

Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
        35                  40                  45

Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
    50                  55                  60

Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
65                  70                  75                  80

Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
                85                  90                  95

Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
            100                 105                 110

Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
        115                 120                 125

Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
    130                 135                 140

Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
145                 150                 155                 160

Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr
                165                 170                 175

Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
            180                 185                 190
```

```
Asn Ala Ser Arg Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser
        195                 200                 205

Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser
    210                 215                 220

Gln His Thr Gln Pro Thr Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser
225                 230                 235                 240

Phe Leu Leu Pro Met Gly Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly
                245                 250                 255

Asp Phe Ala Leu Pro Val Gly Leu Ile Val Gly Val Thr Ala Leu Gly
            260                 265                 270

Leu Leu Ile Ile Gly Val Val Asn Cys Val Ile Met Thr Gln Val Lys
        275                 280                 285

Lys Lys Pro Leu Cys Leu Gln Arg Glu Ala Lys Val Pro His Leu Pro
    290                 295                 300

Ala Asp Lys Ala Arg Gly Thr Gln Gly Pro Glu Gln Gln His Leu Leu
305                 310                 315                 320

Ile Thr Ala Pro Ser Ser Ser Ser Ser Ser Leu Glu Ser Ser Ala Ser
                325                 330                 335

Ala Leu Asp Arg Arg Ala Pro Thr Arg Asn Gln Pro Gln Ala Pro Gly
            340                 345                 350

Val Glu Ala Ser Gly Ala Gly Glu Ala Arg Ala Ser Thr Gly Ser Ser
        355                 360                 365

Asp Ser Ser Pro Gly Gly His Gly Thr Gln Val Asn Val Thr Cys Ile
    370                 375                 380

Val Asn Val Cys Ser Ser Ser Asp His Ser Ser Gln Cys Ser Ser Gln
385                 390                 395                 400

Ala Ser Ser Thr Met Gly Asp Thr Asp Ser Ser Pro Ser Glu Ser Pro
                405                 410                 415

Lys Asp Glu Gln Val Pro Phe Ser Lys Glu Glu Cys Ala Phe Arg Ser
            420                 425                 430

Gln Leu Glu Thr Pro Glu Thr Leu Leu Gly Ser Thr Glu Glu Lys Pro
        435                 440                 445

Leu Pro
    450
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 277 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Val Arg Leu Pro Leu Gln Cys Val Leu Trp Gly Cys Leu Leu Thr
1               5                   10                  15

Ala Val His Pro Glu Pro Pro Thr Ala Cys Arg Glu Lys Gln Tyr Leu
            20                  25                  30

Ile Asn Ser Gln Cys Cys Ser Leu Cys Gln Pro Gly Gln Lys Leu Val
        35                  40                  45

Ser Asp Cys Thr Glu Phe Thr Glu Thr Glu Cys Leu Pro Cys Gly Glu
    50                  55                  60

Ser Glu Phe Leu Asp Thr Trp Asn Arg Glu Thr His Cys His Gln His
65                  70                  75                  80

Lys Tyr Cys Asp Pro Asn Leu Gly Leu Arg Val Gln Gln Lys Gly Thr
                85                  90                  95

Ser Glu Thr Asp Thr Ile Cys Thr Cys Glu Glu Gly Trp His Cys Thr
            100                 105                 110

Ser Glu Ala Cys Glu Ser Cys Val Leu His Arg Ser Cys Ser Pro Gly
        115                 120                 125

Phe Gly Val Lys Gln Ile Ala Thr Gly Val Ser Asp Thr Ile Cys Glu
    130                 135                 140

Pro Cys Pro Val Gly Phe Phe Ser Asn Val Ser Ser Ala Phe Glu Lys
145                 150                 155                 160

Cys His Pro Trp Thr Ser Cys Glu Thr Lys Asp Leu Val Val Gln Gln
                165                 170                 175

Ala Gly Thr Asn Lys Thr Asp Val Val Cys Gly Pro Gln Asp Arg Leu
            180                 185                 190

Arg Ala Leu Val Val Ile Pro Ile Ile Phe Gly Ile Leu Phe Ala Ile
        195                 200                 205

Leu Leu Val Leu Val Phe Ile Lys Lys Val Ala Lys Lys Pro Thr Asn
    210                 215                 220

Lys Ala Pro His Pro Lys Gln Glu Pro Gln Glu Ile Asn Phe Pro Asp
225                 230                 235                 240
```

```
Asp Leu Pro Gly Ser Asn Thr Ala Ala Pro Val Gln Glu Thr Leu His
                245                 250                 255

Gly Cys Gln Pro Val Thr Gln Glu Asp Gly Lys Glu Ser Arg Ile Ser
            260                 265                 270

Val Gln Glu Arg Gln
        275
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 277 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Cys Val Gly Ala Arg Arg Leu Gly Arg Gly Pro Cys Ala Ala Leu
1               5                   10                  15

Leu Leu Leu Gly Leu Gly Leu Ser Thr Val Thr Gly Leu His Cys Val
            20                  25                  30

Gly Asp Thr Tyr Pro Ser Asn Asp Arg Cys Cys His Glu Cys Arg Pro
        35                  40                  45

Gly Asn Gly Met Val Ser Arg Cys Ser Arg Ser Gln Asn Thr Val Cys
    50                  55                  60

Arg Pro Cys Gly Pro Gly Phe Tyr Asn Asp Val Val Ser Ser Lys Pro
65                  70                  75                  80

Cys Lys Pro Cys Thr Trp Cys Asn Leu Arg Ser Gly Ser Glu Arg Lys
                85                  90                  95

Gln Leu Cys Thr Ala Thr Gln Asp Thr Val Cys Arg Cys Arg Ala Gly
            100                 105                 110

Thr Gln Pro Leu Asp Ser Tyr Lys Pro Gly Val Asp Cys Ala Pro Cys
        115                 120                 125

Pro Pro Gly His Phe Ser Pro Gly Asp Asn Gln Ala Cys Lys Pro Trp
    130                 135                 140

Thr Asn Cys Thr Leu Ala Gly Lys His Thr Leu Gln Pro Ala Ser Asn
145                 150                 155                 160
```

Ser Ser Asp Ala Ile Cys Glu Asp Arg Asp Pro Pro Ala Thr Gln Pro
165 170 175

Gln Glu Thr Gln Gly Pro Pro Ala Arg Pro Ile Thr Val Gln Pro Thr
180 185 190

Glu Ala Trp Pro Arg Thr Ser Gln Gly Pro Ser Thr Arg Pro Val Glu
195 200 205

Val Pro Gly Gly Arg Ala Val Ala Ala Ile Leu Gly Leu Gly Leu Val
210 215 220

Leu Gly Leu Leu Gly Pro Leu Ala Ile Leu Leu Ala Leu Tyr Leu Leu
225 230 235 240

Arg Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro Pro Gly Gly
245 250 255

Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp Ala His Ser
260 265 270

Thr Leu Ala Lys Ile
275

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 435 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Leu Leu Pro Trp Ala Thr Ser Ala Pro Gly Leu Ala Trp Gly Pro
1 5 10 15

Leu Val Leu Gly Leu Phe Gly Leu Leu Ala Ala Ser Gln Pro Gln Ala
20 25 30

Val Pro Pro Tyr Ala Ser Glu Asn Gln Thr Cys Arg Asp Gln Glu Lys
35 40 45

Glu Tyr Tyr Glu Pro Gln His Arg Ile Cys Cys Ser Arg Cys Pro Pro
50 55 60

Gly Thr Tyr Val Ser Ala Lys Cys Ser Arg Ile Arg Asp Thr Val Cys
65 70 75 80

Ala Thr Cys Ala Glu Asn Ser Tyr Asn Glu His Trp Asn Tyr Leu Thr

```
                85                  90                  95

Ile Cys Gln Leu Cys Arg Pro Cys Asp Pro Val Met Gly Leu Glu Glu
            100                 105                 110

Ile Ala Pro Cys Thr Ser Lys Arg Lys Thr Gln Cys Arg Cys Gln Pro
        115                 120                 125

Gly Met Phe Cys Ala Ala Trp Ala Leu Glu Cys Thr His Cys Glu Leu
    130                 135                 140

Leu Ser Asp Cys Pro Pro Gly Thr Glu Ala Glu Leu Lys Asp Glu Val
145                 150                 155                 160

Gly Lys Gly Asn Asn His Cys Val Pro Cys Lys Ala Gly His Phe Gln
                165                 170                 175

Asn Thr Ser Ser Pro Ser Ala Arg Cys Gln Pro His Thr Arg Cys Glu
            180                 185                 190

Asn Gln Gly Leu Val Glu Ala Ala Pro Gly Thr Ala Gln Ser Asp Thr
        195                 200                 205

Thr Cys Lys Asn Pro Leu Glu Pro Leu Pro Pro Glu Met Ser Gly Thr
    210                 215                 220

Met Leu Met Leu Ala Val Leu Leu Pro Leu Ala Phe Phe Leu Leu Leu
225                 230                 235                 240

Ala Thr Val Phe Ser Cys Ile Trp Lys Ser His Pro Ser Leu Cys Arg
                245                 250                 255

Lys Leu Gly Ser Leu Leu Lys Arg Arg Pro Gln Gly Glu Gly Pro Asn
            260                 265                 270

Pro Val Ala Gly Ser Trp Glu Pro Pro Lys Ala His Pro Tyr Phe Pro
        275                 280                 285

Asp Leu Val Gln Pro Leu Leu Pro Ile Ser Gly Asp Val Ser Pro Val
    290                 295                 300

Ser Thr Gly Leu Pro Ala Ala Pro Val Leu Glu Ala Gly Val Pro Gln
305                 310                 315                 320

Gln Gln Ser Pro Leu Asp Leu Thr Arg Glu Pro Gln Leu Glu Pro Gly
                325                 330                 335

Glu Gln Ser Gln Val Ala His Gly Thr Asn Gly Ile His Val Thr Gly
            340                 345                 350

Gly Ser Met Thr Ile Thr Gly Asn Ile Tyr Ile Tyr Asn Gly Pro Val
        355                 360                 365

Leu Gly Gly Pro Pro Gly Pro Gly Asp Leu Pro Ala Thr Pro Glu Pro
    370                 375                 380
```

Pro Tyr Pro Ile Pro Glu Glu Gly Asp Pro Gly Pro Pro Gly Leu Ser
385 390 395 400

Thr Pro His Gln Glu Asp Gly Lys Ala Trp His Leu Ala Glu Thr Glu
405 410 415

His Cys Gly Ala Thr Pro Ser Asn Arg Gly Pro Arg Asn Gln Phe Ile
420 425 430

Thr His Asp
435

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGCCCATGGC TTTGCAGATC GCTCCTC 27

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGCAAGCTTT TAGGGCAAGT AAACATG 27

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGCGGATCCG CCATCATGGC CCCGCGCGCC CGGC 34

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCGGTACCT TAGGGCAAGT AAACATG 27

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CGCGGTACCC TGCGAGTTTG AGGAGTG 27

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGCTCTAGAT CAAGCGTAGT CTGGGACGTC GTATGGGTAT TAGGGCAAGT AAACATG 57

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTAAGAAAGC TTTGTACCAG TGAGAAGCAT 30

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GACGTAGTCG ACTCAAGCCT TGGCCCCGCC 30

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCCTACGTCG ACTCAGCTGA CCAATGAGAG AGCATCCT 38

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AACGGCGTCG ACTCAACTGT CCACCTCTTT TTGCAA 36

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CGCTGAGTCG ACTCAGGAGT TACTTGTTTC CAGTCAC 37

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

His Thr Pro His Tyr Pro Glu Gln Glu Thr Glu Pro Pro Leu Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ser Asn Thr Ala Ala Pro Val Gln Glu Thr Leu His Gly Cys Gln
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Asp Ser Leu Pro His Pro Gln Gln Ala Thr Asp Ser Gly His Glu
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Asp Val Thr Thr Val Ala Val Glu Glu Thr Ile Pro Ser Phe Thr
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Glu Tyr Met Asp Arg Pro Ser Gln Pro Thr Asp Gln Leu Leu Phe
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Glu Pro Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala Arg Arg
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Glu Lys Ala Ser Arg Pro Val Gln Glu Gln Gly Gly Ala Lys Ala
1               5                   10                  15
```

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis)*

A. The indications made below relate to the microorganism referred to in the description on page 4, line 21.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution
American Type Culture Collection

Address of depositary institution (*including postal code and country*)

10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit March 13, 1997	Accession Number 97956

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later (*specify the general nature of the indications, e.g., "Accession Number of Deposit"*)

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the TR8 receptor polypeptide having the amino acid sequence at positions from about -25 to about 590 in SEQ ID NO:2;

(b) a nucleotide sequence encoding the TR8 receptor polypeptide having the amino acid sequence at positions from about -25 to about 211 in SEQ ID NO:2;

(c) a nucleotide sequence encoding the TR8 receptor polypeptide having the amino acid sequence at positions from about -5, -3 or +1 to about 590 in SEQ ID NO:2;

(d) a nucleotide sequence encoding the TR8 receptor polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit Number 97956;

(e) a nucleotide sequence encoding the mature TR8 receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit Number 97956;

(f) a nucleotide sequence encoding the TR8 extracellular domain;

(g) a nucleotide sequence encoding the TR8 transmembrane domain;

(h) a nucleotide sequence encoding the TR8 intracellular domain; and

(I) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g) or (h) above.

2. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in SEQ ID NO:1.

3. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in SEQ ID NO:1 encoding a polypeptide having the complete amino acid sequence in SEQ ID NO:2.

4. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in SEQ ID NO:1 encoding the mature TR8 receptor having the mature amino acid sequence in SEQ ID NO:2.

5. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in ATCC Deposit Number 97956.

6. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding a TR8 receptor polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit Number 97956.

7. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the mature TR8 receptor polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit Number 97956.

8. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e), (f), (g) or (h) of claim 1 wherein said polynucleotide which hybridizes does not hybridize under

stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

9. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a TR8 receptor having an amino acid sequence in (a), (b), (c), (d), (e), (f), (g) or (h) of claim 1.

10. The isolated nucleic acid molecule of claim 9, which encodes an epitope-bearing portion of a TR8 receptor polypeptide selected from the group consisting of: a polypeptide comprising amino acid residues from about 10 to about 65 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 82 to about 185 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 211 to about 257 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 267 to about 512 in SEQ ID NO:2; and a polypeptide comprising amino acid residues from about 531 to about 590 in SEQ ID NO:2.

11. The isolated nucleic acid molecule of claim 1, which encodes a TR8 receptor extracellular domain.

12. The isolated nucleic acid molecule of claim 1, which encodes a TR8 receptor transmembrane domain.

13. The isolated nucleic acid molecule of claim 1, which encodes a TR8 receptor intracellular domain.

14. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

15. A recombinant vector produced by the method of claim 14.

16. A method of making a recombinant host cell comprising introducing the isolated nucleic acid molecule of claim 1 into a host cell.

17. A recombinant host cell produced by the method of claim 16.

18. A recombinant method for producing a TR8 polypeptide, comprising culturing the recombinant host cell of claim 17 under conditions such that said polypeptide is expressed and recovering said polypeptide.

19. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence in SEQ ID NO:1 wherein an additional CGC codon is inserted after nucleotide 72 resulting in insertion of an additional R residue after position 3 in SEQ ID NO:2; nucleotide 763 is G instead of A, resulting in the amino acid E instead of F at position 194 in SEQ ID NO:2; and nucleotide 1583 is G instead of T, resulting in the amino acid S instead of I at position 487 in SEQ ID NO:2.

20. An isolated TR8 receptor polypeptide having the amino acid sequence in SEQ ID NO:2 wherein an additional R residue is inserted after position 3 in SEQ ID NO:2; the amino acid at position 194 in SEQ ID NO:2 is E instead of F; and the amino acid at position 487 in SEQ ID NO:2 is S instead of I.

21. An isolated TR8 polypeptide having an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the amino acid sequence of the TR8 polypeptide having the amino acid sequence at positions from about -25 to about 590 in SEQ ID NO:2;

(b) the amino acid sequence of the TR8 polypeptide having the amino acid sequence at positions from about -25 to about 211 in SEQ ID NO:2;

(c) the amino acid sequence of the TR8 polypeptide having the amino acid sequence at positions from about -5, -3 or +1 to about 590 in SEQ ID NO:2;

d) the amino acid sequence of the TR8 polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit Number 97956;

(e) the amino acid sequence of the mature TR8 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit Number 97956;

(f) the amino acid sequence of the TR8 receptor extracellular domain;

(g) the amino acid sequence of the TR8 receptor transmembrane domain;

(h) the amino acid sequence of the TR8 receptor intracellular domain; and

(q) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d), (e), (f), (g) or (h).

22. An isolated polypeptide comprising an epitope-bearing portion of a TR8 receptor protein, wherein said portion is selected from the group consisting of: a polypeptide comprising amino acid residues from about 10 to about 65 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 82 to about 185 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 211 to about 257 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 267 to about 512 in SEQ ID NO:2; and a polypeptide comprising amino acid residues from about 531 to about 590 in SEQ ID NO:2.

23. An isolated antibody that binds specifically to a TR8 receptor polypeptide of claim 21.

24. A method of treating herpes simplex viral infection comprising introducing an effective amount of a soluble fragment of a TR8 polypeptide into an individual to be treated in admixture with a pharmaceutically acceptable carrier.

25. A method of treating a disease state associated with aberrant cell survival comprising introducing an effective amount of a TR8 protein, or agonist or antagonist thereof, into an individual to be treated in admixture with a pharmaceutically acceptable carrier.

26. A method of screening for agonists and antagonists of TR8 activity comprising:

(a) contacting cells which express a TR8 receptor with a candidate compound,

(b) assaying a cellular response, and

(c) comparing the cellular response to a standard cellular response made in absence of the candidate compound; whereby, an increased cellular response over the standard indicates that the compound is an agonist and a decreased cellular response over the standard indicates that the compound is an antagonist.

```
        10                            30                            50
GTCGACCCACGCGTCCGGGCCGCGGCGCCCGCCAGCCTGTCCCGCGCCATGGCCCCGCGC
                                                  M  A  P  R
        70                            90                           110
GCCCGGCGCCGCCCGCTGTTCGCGCTGCTGCTGCTCTGCGCGCTGCTCGCCCGGCTGCAG
A  R  R  R  P  L  F  A  L  L  L  L  C  A  L  L  A  R  L  Q
       130                           150                           170
GTGGCTTTGCAGATCGCTCCTCCATGTACCAGTGAGAAGCATTATGAGCATCTGGGACGG
V  A  L  Q  I  A  P  P  C  T  S  E  K  H  Y  E  H  L  G  R
       190                           210                           230
TGCTGTAACAAATGTGAACCAGGAAAGTACATGTCTTCTAAATGCACTACTACCTCTGAC
C  C  N  K  C  E  P  G  K  Y  M  S  S  K  C  T  T  T  S  D
       250                           270                           290
AGTGTATGTCTGCCCTGTGGCCCGGATGAATACTTGGATAGCTGGAATGAAGAAGATAAA
S  V  C  L  P  C  G  P  D  E  Y  L  D  S  W  N  E  E  D  K
       310                           330                           350
TGCTTGCTGCATAAAGTTTGTGATACAGGCAAGGCCCTGGTGGCCGTGGTCGCCGGCAAC
C  L  L  H  K  V  C  D  T  G  K  A  L  V  A  V  V  A  G  N
       370                           390                           410
AGCACGACCCCCCGGCGCTGCGCGTGCACGGCTGGGTACCACTGGAGCCAGGACTGCGAG
S  T  T  P  R  R  C  A  C  T  A  G  Y  H  W  S  Q  D  C  E
       430                           450                           470
TGCTGCCGCCGCAACACCGAGTGCGCGCCGGGCCTGGGCGCCCAGCACCCGTTGCAGCTC
C  C  R  R  N  T  E  C  A  P  G  L  G  A  Q  H  P  L  Q  L
       490                           510                           530
AACAAGGACACAGTGTGCAAACCTTGCCTTGCAGGCTACTTCTCTGATGCCTTTTCCTCC
N  K  D  T  V  C  K  P  C  L  A  G  Y  F  S  D  A  F  S  S
       550                           570                           590
ACGGACAAATGCAGACCCTGGACCAACTGTACCTTCCTTGGAAAGAGAGTAGAACATCAT
T  D  K  C  R  P  W  T  N  C  T  F  L  G  K  R  V  E  H  H
       610                           630                           650
GGGACAGAGAAATCCGATGTGGTTTGCAGTTCTTCTCTGCCAGCTAGAAAACCACCAAAT
G  T  E  K  S  D  V  V  C  S  S  S  L  P  A  R  K  P  P  N
       670                           690                           710
GAACCCCATGTTTACTTGCCCGGTTTAATAATTCTGCTTCTCTTCGCGTCTGTGGCCCTG
E  P  H  V  Y  L  P  G  L  I  I  L  L  L  F  A  S  V  A  L
       730                           750                           770
GTGGCTGCCATCATCTTTGGCGTTTGCTATAGGAAAAAAGGGAAAGCACTCACAGCTAAT
V  A  A  I  I  F  G  V  C  Y  R  K  K  G  K  A  L  T  A  N
       790                           810                           830
TTGTGGCACTGGATCAATGAGGCTTGTGGCCGCCTAAGTGGAGATAAGGAGTCCTCAGGT
L  W  H  W  I  N  E  A  C  G  R  L  S  G  D  K  E  S  S  G
       850                           870                           890
GACAGTTGTGTCAGTACACACACGGCAAACTTTGGTCAGCAGGGAGCATGTGAAGGTGTC
D  S  C  V  S  T  H  T  A  N  F  G  Q  Q  G  A  C  E  G  V
       910                           930                           950
TTACTGCTGACTCTGGAGGAGAAGACATTTCCAGAAGATATGTGCTACCCAGATCAAGGT
L  L  L  T  L  E  E  K  T  F  P  E  D  M  C  Y  P  D  Q  G
       970                           990                          1010
GGTGTCTGTCAGGGCACGTGTGTAGGAGGTGGTCCCTACGCACAAGGCGAAGATGCCAGG
G  V  C  Q  G  T  C  V  G  G  G  P  Y  A  Q  G  E  D  A  R
```

FIG.1A

```
       1030                           1050                           1070
ATGCTCTCATTGGTCAGCAAGACCGAGATAGAGGAAGACAGCTTCAGACAGATGCCCACA
M  L  S  L  V  S  K  T  E  I  E  E  D  S  F  R  Q  M  P  T
       1090                           1110                           1130
GAAGATGAATACATGGACAGGCCCTCCCAGCCCACAGACCAGTTACTGTTCCTCACTGAG
E  D  E  Y  M  D  R  P  S  Q  P  T  D  Q  L  L  F  L  T  E
       1150                           1170                           1190
CCTGGAAGCAAATCCACACCTCCTTTCTCTGAACCCCTGGAGGTGGGGGAGAATGACAGT
P  G  S  K  S  T  P  P  F  S  E  P  L  E  V  G  E  N  D  S
       1210                           1230                           1250
TTAAGCCAGTGCTTCACGGGGACACAGAGCACAGTGGGTTCAGAAAGCTGCAACTGCACT
L  S  Q  C  F  T  G  T  Q  S  T  V  G  S  E  S  C  N  C  T
       1270                           1290                           1310
GAGCCCCTGTGCAGGACTGATTGGACTCCCATGTCCTCTGAAAACTACTTGCAAAAAGAG
E  P  L  C  R  T  D  W  T  P  M  S  S  E  N  Y  L  Q  K  E
       1330                           1350                           1370
GTGGACAGTGGCCATTGCCCGCACTGGGCAGCCAGCCCCAGCCCCAACTGGGCAGATGTC
V  D  S  G  H  C  P  H  W  A  A  S  P  S  P  N  W  A  D  V
       1390                           1410                           1430
TGCACAGGCTGCCGGAACCCTCCTGGGGAGGACTGTGAACCCCTCGTGGGTTCCCCAAAA
C  T  G  C  R  N  P  P  G  E  D  C  E  P  L  V  G  S  P  K
       1450                           1470                           1490
CGTGGACCCTTGCCCCAGTGCGCCTATGGCATGGGCCTTCCCCCTGAAGAAGAAGCCAGC
R  G  P  L  P  Q  C  A  Y  G  M  G  L  P  P  E  E  E  A  S
       1510                           1530                           1550
AGGACGGAGGCCAGAGACCAGCCCGAGGATGGGGCTGATGGGAGGCTCCCAAGCTCAGCG
R  T  E  A  R  D  Q  P  E  D  G  A  D  G  R  L  P  S  S  A
       1570                           1590                           1610
AGGGCAGGTGCCGGGTCTGGAATCTCCCCTGGTGGCCAGTCCCCTGCATCTGGAAATGTG
R  A  G  A  G  S  G  I  S  P  G  G  Q  S  P  A  S  G  N  V
       1630                           1650                           1670
ACTGGAAACAGTAACTCCACGTTCATCTCCAGCGGGCAGGTGATGAACTTCAAGGGCGAC
T  G  N  S  N  S  T  F  I  S  S  G  Q  V  M  N  F  K  G  D
       1690                           1710                           1730
ATCATCGTGGTCTACGTCAGCCAGACCTCGCAGGAGGGCGCGGCGGCGGCTGCGGAGCCC
I  I  V  V  Y  V  S  Q  T  S  Q  E  G  A  A  A  A  A  E  P
       1750                           1770                           1790
ATGGGCCGCCCGGTGCAGGAGGAGACCCTGGCGCGCCGAGACTCCTTCGCGGGGAACGGC
M  G  R  P  V  Q  E  E  T  L  A  R  R  D  S  F  A  G  N  G
       1810                           1830                           1850
CCGCGCTTCCCGGACCCGTGCGGCGGCCCCGAGGGGCTGCGGGAGCCGGAGAAGGCCTCG
P  R  F  P  D  P  C  G  G  P  E  G  L  R  E  P  E  K  A  S
       1870                           1890                           1910
AGGCCGGTGCAGGAGCAAGGCGGGGCCAAGGCTTGAGCGCCCCCCATGGCTGGGAGCCCG
R  P  V  Q  E  Q  G  G  A  K  A  *
       1930                           1950                           1970
AAGCTCGGAGCCAGGGCTCGCGAGGGCAGCACCGCAGCCTCTGCCCCAGCCCCGGCCACC
       1990                           2010                           2030
CAGGGATCGATCGGTACAGTCGAGGAAGACCACCCGGCATTCTCTGCCCACTTTGCCTTC
       2050                           2070                           2090
CAGGAAATGGGCTTTTCAGGAAGTGAATTGATGAGGACTGTCCCCATGCCCACGGATGCT
       2110                           2130                           2150
CAGCAGCCCGCCGCACTGGGGCAGATGTCTCCCCTGCCACTCCTCAAACTCGCAGCAGTA
```

FIG.1B

```
    2170                      2190                      2210
ATTTGTGGCACTATGACAGCTATTTTTATGACTATCCTGTTCTGTGGGGGGGGGGGTCTG
    2230                      2250                      2270
TTTTCCCCCCATATTTGTATTCCTTTTCATAACTTTTCTTGATATCTTTCCTCCCTCTTT
    2290                      2310                      2330
TTTAATGTAAAGGTTTTCTCAAAAATTCTCCTAAAGGTGAGGGTCTCTTTCTTTTCTCTT
    2350                      2370                      2390
TTCCTTTTTTTTTTCTTTTTTTGGCAACCTGGCTCTGGCCCAGGCTAGAGTGCAGTGGTG
    2410                      2430                      2450
CGATTATAGCCCGGTGCAGCCTCTAACTCCTGGGCTCAAGCAATCCAAGTGATCCTCCCA
    2470                      2490                      2510
CCTCAACCTTCGGAGTAGCTGGGATCACAGCTGCAGGCCACGCCCAGCTTCCTCCCCCCG
    2530                      2550                      2570
ACTCCCCCCCCAGAGACACGGTCCCACCATGTTAACCCAGCCTGGTCTCAAACTCACCCA
    2590                      2610                      2630
GTAAAGCAGTCCTACCAGCCTCGGCCTCCCAAAGTCACTGGGATTCACAGGCGTGAGCCC
    2650                      2670                      2690
CCACGCTGGCCTGCTTTACGTATTTTCTTTTGTGCCCCTGCTCACAGTGTTTTAGAGATG
    2710                      2730                      2750
GCTTTCCCAGTGTGTGTTCATTGTAAACACTTTTGGGAAAGGGCTAAACATGTGAGGCCT
    2770                      2790                      2810
GGAGATAGTTGCTAAGTTGCTAGGAACATGTGGTGGGACTTTCATATTCTGAAAAATGTT
    2830                      2850
CTATATTCTCATTTTTCTAAAAAAAAAAAAAAA
```

FIG.1C

4/9

```
  1 MAPRARRRPL.FALLLLCALLA.RLQVAL.....QIAPPCTSEKHYEHLG  43
  1 mapvavwaalavglelwaaahalpaqvaftpyapepgstcrlreyydqta  50

 44 R.CCNKCEPGKYMSSKCTTTSDSVCLPCGPDEYLDSWNEEDKCLLHKVCD  92
 51 qmccskcspgqhakvfctktsdtvcdscedstytqlwnwvpeclsc.gsr  99

 93 TGKALVAVVAGNSTTPRRCACTAGYH....WSQDCECCRRNTECAPGLGA 138
100 cssdqvetqactreqnrictcrpgwycalskqegcrlcaplrkcrpgfgv 149

139 QHPLQLNKDTVCKPCLAGYFSDAFSSTDKCRPWTNCTFLGKRVEHHGTEK 188
150 arpgtetsdvvckpcapgtfsnttsstdicrphqicnv....vaipgnas 195

189 SDVVCSSSLPARKPPNEPHVYLPGLIILLLFASVALVAAIIFGVCYRKKG 238
196 rdavctstsptrsmapga.vhlpqpv........................ 220

239 KALTANLWHWINEACGRLSGDKESSGDSCVSTHTANFGQQG.ACEGVLLL 287
221 ...........................strsqhtqptpepstapstsfll 243

288 TLEEKTFPEDMCYPDQGGVCQGTCVGGGPYAQGEDARMLSLVSKTEIEED 337
244 pmg............................................... 246

338 SFRQMPTEDEYMDRPSQPTDQLLFLTEPGSKSTPPFSEPLEVGENDSLSQ 387
247 ..............psppae........gstgdfalpvglivgvtalgll 274

388 CFTGTQSTVGSESCNCTEPLCRTDWTPMSSENYLQKEVDSGHCPHWAASP 437
275 iigvvncvi..........mtqvkkkpl....clqreakvphlp...... 304

438 SPNWADVCTGCRNPPGEDCEPLVGSPKRGPLPQCAYGMGLPPEEEASRTE 487
305 ....adkargtqgpeqqhllitapsssssslessasald.......rrap 343

488 ARDQPED.GADGRLPSSARAGAGSGISPGGQSPASGNVTGNSNSTFISSG 536
344 trnqpqapgveasgagearastgssdsspgghgtqvnvtcivn...vcss 390

537 QVMNFKGDIIVVYVSQTSQEGAAAAAEPMGRPVQEETLARRDSFAGNGPR 586
391 ......sdhssqcssqasstmgdtdsspsespkdeqv.....pfskeeca 429

587 FPDPCGGPEGLREP..EKASRPVQEQGGAKA 615
430 frsqletpetllgsteekplplgvpdagmkp 460
```

FIG.2

```
  1  MA-------PVAVWAALAVGLELWAAAHALPAQVAFTPYAPEPGSTCR--  h TNFR 2
  1  MV-R---------L-PLQCVL---WGCLLTAVHPEPPT-------ACREK  h CD40
  1  MCVGARRLGRGPCAALLLLGLGL---STVTGLHCVGDTYPSN--------  h OX40
  1  MLLPWATSAPGLAWGPLVLGL---FGLLAASQPQAVPPYASE-NQTCRDQ  h LTbetaR
  1  MA-PRARRRPLFAL-LLLCAL---LARLQVALQIAPPC-------TS-EK  HDPIK17XXb protein

 42  LREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYTQLWNWVP  h TNFR 2
 30  Q---YLINSQ-CCSLCQPGQKLVSDCTEFTETECLPCGESEFLDTWNRET  h CD40
 40  ---------DRCCHECRPGNGMVSRCSRSQNTVCRPCGPGFYNDVVSSKP  h OX40
 47  EKEYYEPQHRICCSRCPPGTYVSAKCSRIRDTVCATCAENSYNEHWNYLT  h LTbetaR
 38  H---YEHLGR-CCNKCEPGKYMSSKCTTTSDSVCLPCGPDEYLDSWNEED  HDPIK17XXb protein

 92  ECLSCGSRCSSDQV--ETQACTREQNRICTCRPGWYCALSKQEGCRLCAP  h TNFR 2
 76  HCHQH-KYCDPNLGLRVQQKGTSETDTICTCEEGWHC--TSEA-CESCVL  h CD40
 81  -CKPCTW-CNLRSGSERKQLCTATQDTVCRCRAG----------------  h OX40
 97  ICQLC-RPCDPVMGLEEIAPCTSKRKTQCRCQPGMFCAAWALE-CTHCEL  h LTbetaR
 84  KCLLH-KVCDTGKALVAVVAGNSTTPRRCACTAGYH---WSQD-CECCRR  HDPIK17XXb protein

140  LRKCRPGFGVARPGT-ETSDVVCKPCAPGTFSNTTSSTDICRPHQICN--  h TNFR 2
122  HRSCSPGFGVKQIATGVS-DTICEPCPVGFFSNVSSAFEKCHPWTSCETK  h CD40
113  ----------TQPLDSYKPGVDCAPCPPGHFS--PGDNQACKPWTNCTLA  h OX40
145  LSDCPPGTEAELKDEVGKGNNHCVPCKAGHFQNTSSPSARCQPHTRCENQ  h LTbetaR
129  NTECAPGLGAQHPLQLNK-DTVCKPCLAGYFSDAFSSTDKCRPWTNCTFL  HDPIK17XXb protein

187  --VVAIPGNASRDAVCTST-SPTRSMAPGAVHLPQPV-------------  h TNFR 2
171  DLVVQQAGTNKTDVVCG-----------PQDRLRALVVI----------P  h CD40
151  GKHTLQPASNSSDAICEDRDPPATQPQETQGPPARPITVQ----------  h OX40
195  GLVEAAPGTAQSDTTCKNPLEPL-PPEMSGTML--MLAVLLPLAFFLLLA  h LTbetaR
178  GKRVEHHGTEKSDVVCSSSLPARKPPNEPHVYLPGLIILLLFASVALVAA  HDPIK17XXb protein
```

FIG.3A

```
221 --------------------------STRSQHTQPTPEPSTAPSTS---- h TNFR 2
200 IIFGILF------------------------------------------- h CD40
191 --------------PTEAWPRTSQ-----GPSTRPVEVPGGRAVAAILGL h OX40
242 TVFSC------------IWKSHPSLCRKLGS------------------- h LTbetaR
228 IIFGVCYRKKGKALTANLWHWINEACGRLSGDKESSGDSCVSTHTANFGQ HDPIK17XXb protein

241 -------FLLPM-----------------------GPSPPAEG------- h TNFR 2
207 -----AILLV--------------------------------------LV h CD40
222 G------LVLGL-----------------------------LGPLAILLA h OX40
261 -------LL----------------------------------------- h LTbetaR
278 QGACEGVLLLTLEEKTFPEDMCYPDQGGVCQGTCVGGGPYAQGEDARMLS HDPIK17XXb protein

254 ---------------------------STGDFALPVGLIVGVTALGL--- h TNFR 2
214 FIKKVA-------KKPTNKA--PHPKQEPQEINFPDDLPGSNTAAPVQET h CD40
237 L-------------------YL---------------------------- h OX40
263 ------------KRRPQGE------------------------------- h LTbetaR
328 LVSKTEIEEDSFRQMPTEDEYMDRPSQPTDQLLFLTE-PGSKSTPPFSEP HDPIK17XXb protein

274 LIIG---VVNCVIMTQVK---------KKPLC------------LQREAK h TNFR 2
255 LH------------------------------------------------ h CD40
240 ------------------------------LRRDQRLPPDAH-------- h OX40
270 --------------GPNPVAGS-------------WEPPKAHPYF----- h LTbetaR
377 LEVGENDSLSQCFTGTQSTVGSESCNCTEPLCRTDWTPMSSENYLQKEVD HDPIK17XXb protein
```

FIG.3B

7/9

```
300 V P H L P - - - - - - - - - - A D K A R G T Q G P E Q Q H L L I T A P S S S S S L E S S A S A L D   h TNFR 2
257 - - - - - - - - - - - - - - - - - - - - - - - - - - - - G C Q P V T - - - - - - - - - - - - - - - -   h CD40
252 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   h OX40
288 - - - - P D L V Q P L L P I S G D V S P V S T G L P A A P V L E A G V P Q Q Q S P L - - - - - - - D   h LTbetaR
427 S G H C P H W A A S P S P N W A D V C T G C R N P P G E D C E P L V G S P K R G P L P Q C A Y G M G   HDPIK17XXb protein

340 - - - - - - R R A P T R N Q P Q A P G V E A S G A G E A R A S T G S S - - - - - - - - - - D S S P G   h TNFR 2
263 - - - - - - - - - - - - - - - Q E D G K E S R I S V Q E R - - - - - - - - - - - - - - - - - - - - -   h CD40
252 - - - - - - - - - - - - - - - - - - - - - - - - - - - - K P P G G G S F - - - - - - - - - - - - - -   h OX40
327 L T R E P Q L E P G E Q S Q V A H G T N G I H V T G G S M T I T G N I Y I Y N G P V L G G P P G P G   h LTbetaR
477 L P P E E E A S R T E A R D Q P E D G A D G R L P S S A R A G A G S G - I S P G - - - G Q S P A S G   HDPIK17XXb protein

374 G H G T Q V N V T C I V N - - - V C S S S D H S S Q C S S Q A S S T M G D T D S S P S E S P K D E Q   h TNFR 2
277 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   h CD40
260 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - R T P I Q E E   h OX40
377 D L P A T P E P P Y - - - - - - - - - - - - - - - - - - - P I P E E G D P G P P G - L S T P H Q E D   h LTbetaR
523 N V T G N S N S T F I S S G Q V M N F K G D I I V V Y V S Q T S Q E G A A A A A E P M G R P V Q E E   HDPIK17XXb protein

421 V - - - - - P F S K E E C A F R S Q L E T P E T L L G S T E E K P L P L G V P D A G M K P S   h TNFR 2
277 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - Q   h CD40
267 Q A D A H S T L A K I   h OX40
407 G K A W H - - L A E T - - - - - E H C G A T P S N R G P - - R N Q F - - - - - - - - I T H D   h LTbetaR
573 T L A R R D S F A G N G P R F P D P C G G P E G L R E P - - E K A S R P V Q E Q G G A K A   HDPIK17XXb protein
```

FIG.3C

8/9

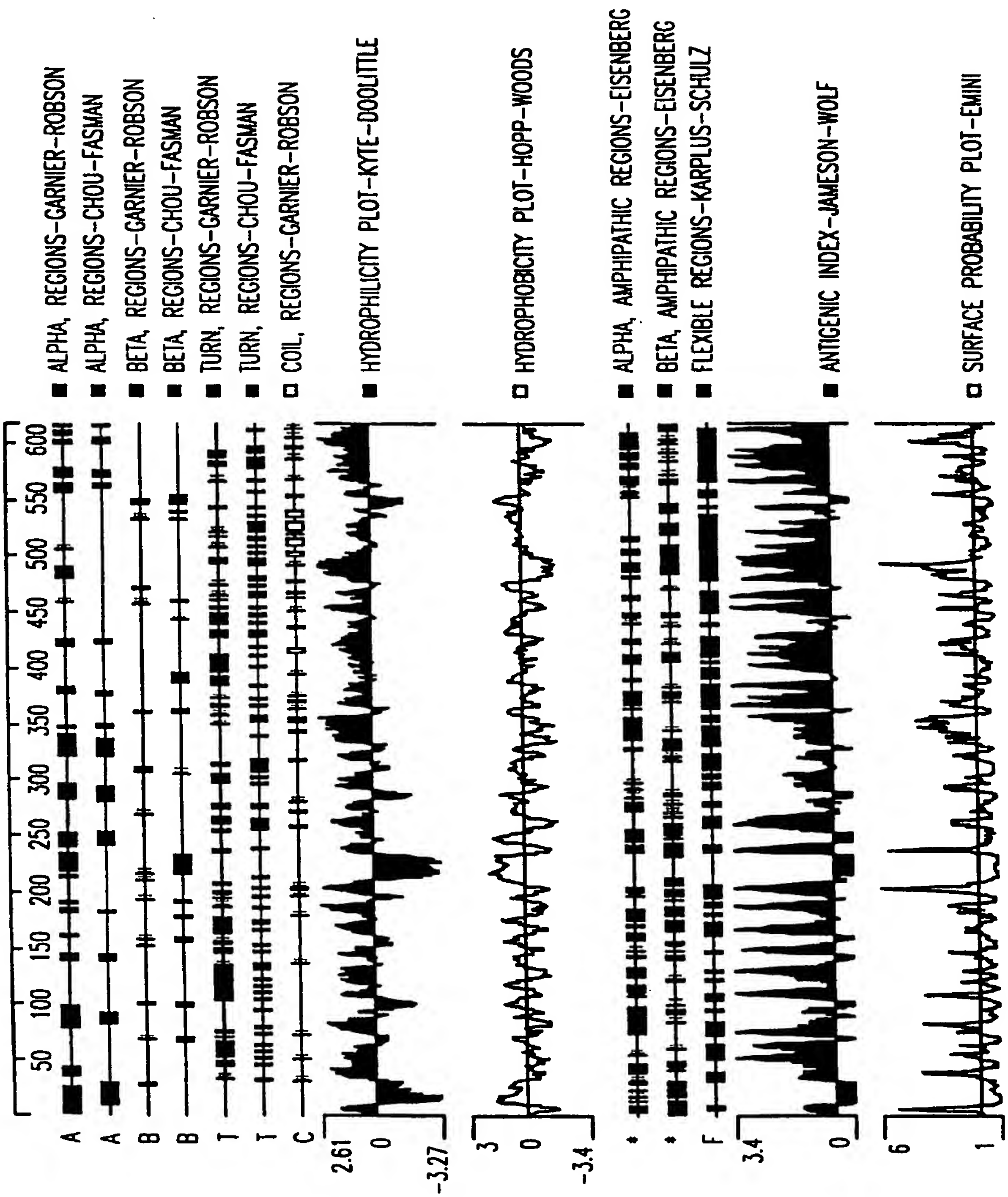
ALPHA, REGIONS-GARNIER-ROBSON
ALPHA, REGIONS-CHOU-FASMAN
BETA, REGIONS-GARNIER-ROBSON
BETA, REGIONS-CHOU-FASMAN
TURN, REGIONS-GARNIER-ROBSON
TURN, REGIONS-CHOU-FASMAN
COIL, REGIONS-GARNIER-ROBSON
HYDROPHILICITY PLOT-KYTE-DOOLITTLE
HYDROPHOBICITY PLOT-HOPP-WOODS
ALPHA, AMPHIPATHIC REGIONS-EISENBERG
BETA, AMPHIPATHIC REGIONS-EISENBERG
FLEXIBLE REGIONS-KARPLUS-SCHULZ
ANTIGENIC INDEX-JAMESON-WOLF
SURFACE PROBABILITY PLOT-EMINI
50
100
150
200
250
300
350
400
450
500
550
600
A
A
B
B
T
T
C
2.61
0
-3.27
3
0
-3.4
F
3.4
0
6
1

FIG.4

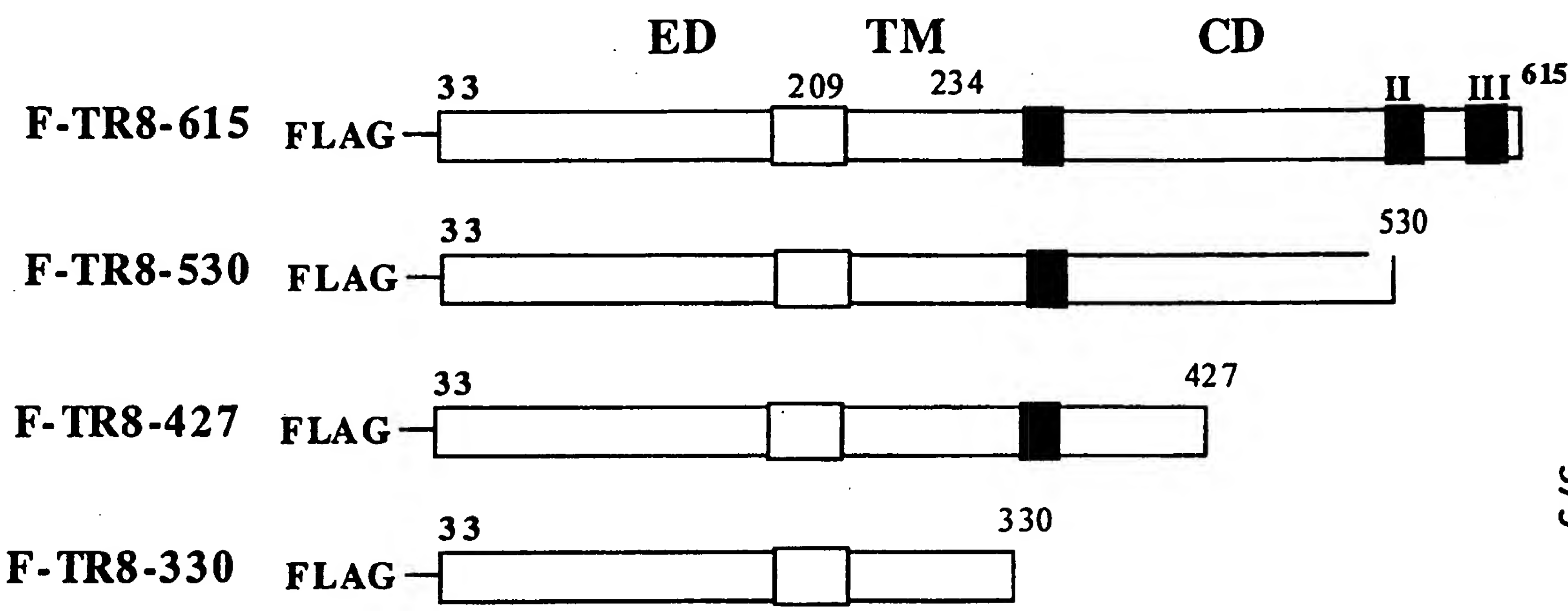

FIG. 5A

CD30	556	H	T	P	H	Y	**P**	E	**Q**	**E**	**T**	E	P	P	L	G	(SEQ ID NO: 18)
CD40	245	S	N	T	A	A	**P**	**V**	**Q**	**E**	**T**	L	H	G	C	Q	(SEQ ID NO: 19)
LMP-1	199	D	S	L	P	H	**P**	Q	**Q**	A	**T**	D	S	G	H	E	(SEQ ID NO: 20)
ATAR/HVEM	263	D	V	T	T	V	A	**V**	E	**E**	**T**	I	P	S	F	T	(SEQ ID NO: 21)
hTR8(I)	322	E	Y	M	D	R	**P**	S	**Q**	P	**T**	D	Q	L	L	F	(SEQ ID NO: 22)
hTR8(II)	538	E	P	M	G	R	**P**	**V**	**Q**	**E**	E	T	L	A	R	R	(SEQ ID NO: 23)
hTR8(III)	576	E	K	A	S	R	**P**	**V**	**Q**	**E**	Q	G	G	A	K	A	(SEQ ID NO: 24)

FIG. 5B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/10980

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/04; C07K 14/705; C12N 15/09, 15/63; C12Q 1/68
US CL : 536/23.5, 24.3; 435/7.2, 69.1, 320.1; 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5, 24.3; 435/7.2, 69.1, 320.1; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	ANDERSON, D.M et al. A Homologue of the TNF Receptor and its Ligand Enhance T-Cell Growth and Dendritic-Cell Function. Nature, 13 November 1997. Vol. 390, pages 175-179.	1-24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:
"A"	document defining the general state of the art which is not considered to be of particular relevance
"E"	earlier document published on or after the international filing date
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed
"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 AUGUST 1998	10 SEP 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Authorized officer
NIRMAL S. BASI
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/10980

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-24

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, MEDLINE, JAPIO, BIOSIS, SCISEARCH, WPIDS, EMBSE, SPTREMB15, N-GENESEQ31-2, EMBL-EST54, PIR56, SWISS-PROT35
tumor necrosis factor, cd40 protein, ox40 protein, lymphotoxin beta recepror, herpes simplex virus, SEQ ID NO:1 and 2

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-24, drawn to: a)nucleic acid encoding the TR8 receptor polynucleotide; b) nucleotide sequences which hybridize to said nucleic acid; c) recombinant vectors and host cells containing said nucleic acid and producing TR8 protein; d) method of treating herpes simplex viral infection using a soluble fragment of a TR8 polypeptide.
Group II, claim 25, drawn to a method of treatment of a disease state associated with aberrant cell survival comprising introducing an effective amount of a TR8 protein, or agonist or antagonist thereof.
Group III, claim 26, drawn to method of screening for antagonists and agonist of TR8 activity.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Group I is directed to nucleic acid encoding the TR8 receptor polypeptide, nucleotide sequences which hybridize to said nucleic acid, recombinant vectors, host cells for producing TR8 protein, and method of treating herpes simplex viral infection, which is the first appearing product, method of making and method of using. The special technical feature is the disclosed nucleic acid sequence of TR8 receptor. Group II and III are drawn to methods having different goals and method steps which do not share the same technical feature with Group I.
Therefore, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.